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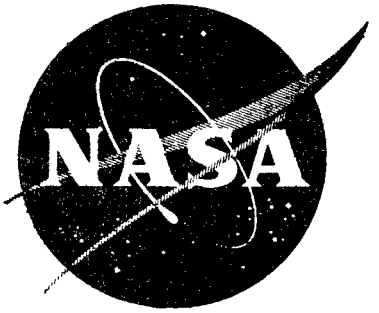
TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1967

E. J. FAIRCHILD II

Aerojet-General Corporation

DECEMBER 1967

JOINT NASA/USAF STUDY



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(U) This report reviews the activities of the Toxic Hazards Research Unit (THRU) since August, 1966. Included is a brief resume of various facility and equipment design modifications which have occurred; in addition, research accomplishments are reported and pertain to all experiments conducted during the year, including those which were begun during the period of the last annual report. (Author)

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FOREWORD

This is the third annual report of activities of the Toxic Hazards Research Unit laboratory and concerns work conducted by Aerojet-General Corporatioon on behalf of the Air Force under Contract No. F33615-67-C-1025. The report also encompasses research performed previously under Contract AF 33(657)-11305. These ongoing activities are appropriately indicated in the preceding annual report (AMRL-TR-66-177). This report covers work from September, 1966 to August, 1967.

The contract for the laboratory facilities and their operation was initiated in 1963 under Project No. 6302, "Toxic Hazards of Propellants and Materials," and Task No. 630201, "Toxicology". K. C. Back, Ph.D., Chief of the Toxicology Branch, is the contract monitor for the Aerospace Medical Research Laboratories.

J. D. MacEwen, Ph.D., of Aerojet-General Corporation, served as principal investigator and Laboratory Director until January, 1967, at which time this responsibility was assumed by E. J. Fairchild II, Ph.D. Significant contributions for responsibility of activities covered by this report were made by Mr. E. H. Vernot, current Assistant Laboratory Director, and Mr. C. E. Johnson, Facility Engineer, both of Aerojet-General Corporation. The National Aeronautics and Space Administration provided support for the Apollo Materials Toxicity Screening Program.

This report is designated as Aerojet-General Report No. 3453.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

AMRL-TR-67-137

TOXIC HAZARDS RESEARCH UNIT ANNUAL TECHNICAL REPORT: 1967

E. J. FAIRCHILD II

AMRL-TR-67-175

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FOREWORD

This study was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry." The work was performed from September 1965 to September 1966 in the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory. Valuable assistance rendered by Major Ralph Ziegler, Major Vernon Carter, Captain Gale Taylor and Miss Marilyn George is gratefully acknowledged.

The material presented in this Technical Documentary Report was presented at the Society of Toxicology Sixth Annual Meeting on 25 March 1967 in Atlanta, Georgia.

This technical report has been reviewed and is approved.

WAYNE H. McCANDLESS
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT

This report reviews the activities of the Toxic Hazards Research Unit (THRU) since August, 1966. Included is a brief resume' of various facility and equipment design modifications which have occurred; in addition, research accomplishments are reported and pertain to all experiments conducted during the year, including those which were begun during the period of the last annual report. Facility modifications have primarily been restricted to the Altitude Facility with the view in mind to assume greater safety against fire hazards in environments of high oxygen concentrations. Toward this end, much has been accomplished in design and test of fire extinguishing systems. Research activities were curtailed by the restrictions placed upon use of environmental atmospheres containing oxygen concentrations greater than ambient; however, various control studies were designed and conducted to take advantage of the abated experimental programs. An eight-month study of the effects of a mixed gas (oxygen-nitrogen) atmosphere at 5 psia indicated some adverse findings. The Apollo materials toxicity screening tests have continued with little or no evidence of toxicity exhibited by their gas-off products. Special studies, including one with saturated concentrations of ethylene glycol, were conducted and the findings are reported herein.

Ambient Facility experiments have mainly comprised work with monomethylhydrazine and certain control studies to delineate improvement of animal sacrifice techniques. Monomethylhydrazine exposures of various time periods are reported for mice, rats, dogs and monkeys.

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SECTION I

INTRODUCTION

The Toxic Hazards Research Unit (THRU), operated by Aerojet-General Corporation personnel for the Air Force is employed in the general implementation of toxicologic investigations. The nature of this research is dictated by the desire of the Air Force to better delineate problems, as well as solutions to the problems, that may arise from either accidental or unavoidable exposure of personnel to materials having acute or potentially chronic toxic effects. To this end, agents that are potential exposure hazards can be tested for their effects on animals under ambient conditions of pressure and atmosphere. Similarly, animal experimentation in support of manned space flight programs can be conducted with simulation of atmospheric conditions in space cabins.

Accordingly, the THRU facility conducts a continuing research program involving, either directly or indirectly through Air Force support, all disciplines of inhalation toxicology, e.g., analytical chemistry, clinical chemistry, engineering, and biological sciences. Included in the facility for accomplishment of inhalation exposures are: preconditioning chambers for animal control studies; Rochester and Longley chambers for animal exposures to atmospheric contaminants under conditions of ambient air and pressure; and altitude chambers (designated as Thomas Domes) for exposure of animals to space cabin environments, with or without controlled contaminant. The latter chamber units, referred to herein as the Altitude Facility, can be used to investigate problems associated with single-gas atmospheres (100% oxygen), two-gas atmospheres (varying mixtures of oxygen and nitrogen), or multiple-gas atmospheres, at desired pressure (altitude) and contaminant levels. Greater detail of the design and operation of the THRU facility is described in references 7 and 8.

This report summarizes research accomplishments and various facility and equipment design modifications conducted since the last annual report (Reference 8), and includes the period from September 1966 through August 1967. The report also covers the annual toxicology conference, plans for work in the immediate future, and the status of programs in progress.

SECTION II

FACILITIES

GENERAL

This section presents various activities of the facility units during the period covered by this report, in relation to the importance of maintenance and backup for actual experimentation. Most of the salient features and associated problems of design changes, equipment changes or additions, and chemistry methodology and instrumentation are included. Schematics of design changes cover only those alterations made since the prior reports, e.g., AMRL-TR-65-125 (Reference 7) pertaining to details of the animal exposure facilities and related equipment, and AMRL-TR-66-177 (Reference 8), the 1966 annual technical report.

A. ENGINEERING FACILITY

During the past year, the THRU facility has established a Facility Engineering Department. The department provides support to all phases of experimental operations. Emphasis has been placed increasingly on greater capability in various areas. The personnel and equipment acquired permit the performance of nearly all support requirements of the Thomas Domes. Preventive and corrective maintenance procedures have been formulated, and Facility Engineering personnel are assigned to each shift to provide immediate emergency assistance.

The following is a list of jobs or job objectives that either have been completed, are in the process of being completed, or are proposed:

1. A modification to the emergency contaminant solenoid system has been completed (Figure 1). The previous method of venting contaminants in case of emergency conditions was by an electrical three-way solenoid, activated manually by the chamber operator. This solenoid was installed in the dome input line prior to its penetration of the dome floor. Several problems related to this system are cited below as examples:

   a. Manual Activation of Contaminant Solenoid

   During emergency conditions, it is preferable to limit the number of actions required of the chamber operator. If the contaminant is not vented during dome isolation, high contaminant concentrations will ensue with subsequent risk of overexposure of experimental animals.

   b. High Contaminant Concentrations on Restoration of Contaminant Flow to Domes

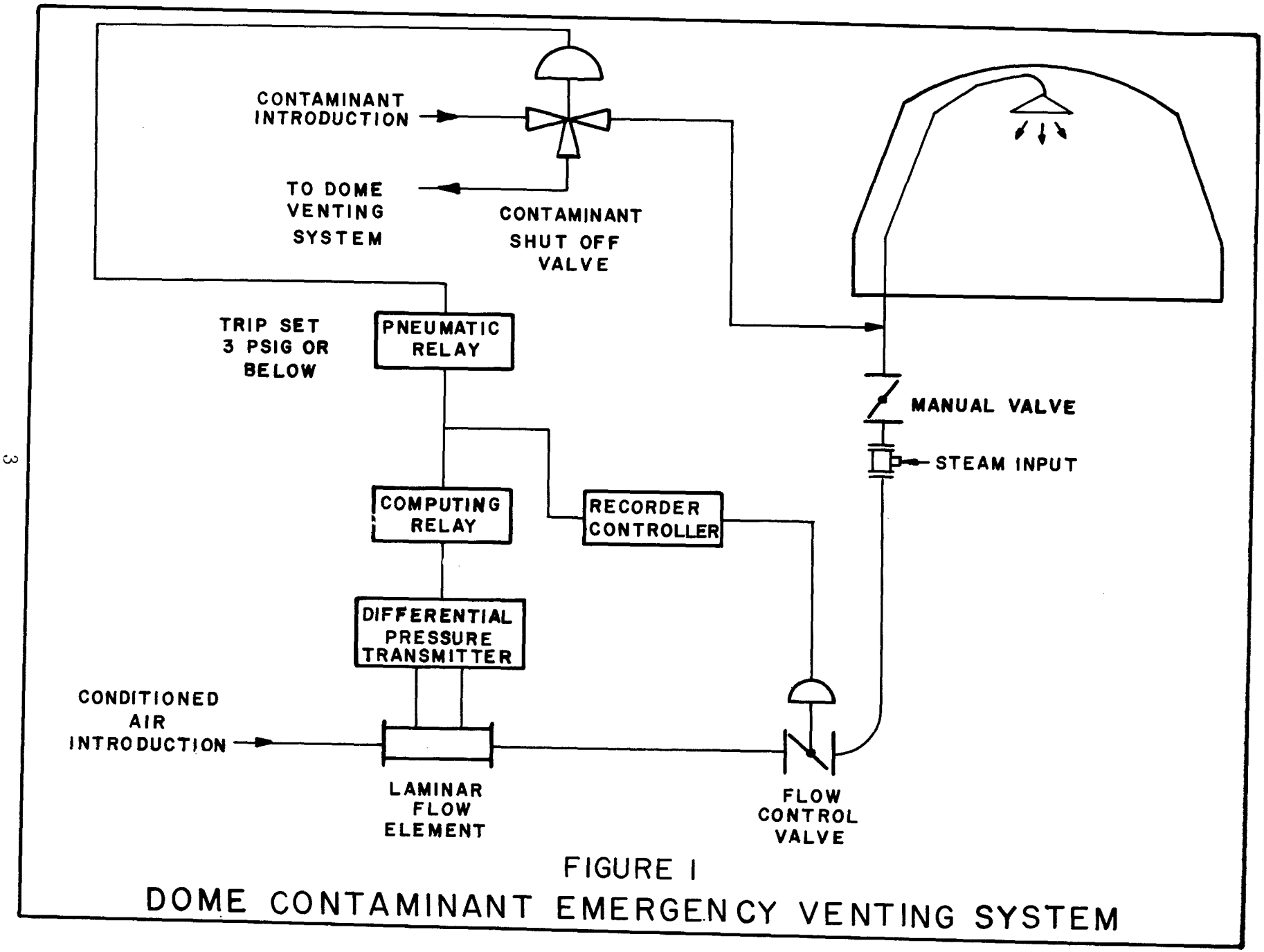


FIGURE 1

DOME CONTAMINANT EMERGENCY VENTING SYSTEM

When the domes are operating under negative pressure conditions and the contaminant is vented, the contaminant feed lines from the generating area rise to ambient conditions. As these lines contain a significant volume, when the contaminant flow is returned to the dome, high concentrations occur temporarily. This has been compensated for in the past by increasing dome flow to 60 cubic feet per minute (cfm) until the concentration reaches a safe level.

c. Contaminant Venting Protection for All Conditions

At present, if the chamber operator is temporarily absent, a high contaminant concentration may occur in the domes before the contaminant can be vented.

The foregoing problems have been alleviated in the modified system. When dynamic flow conditions are maintained in the domes, the contaminant concentration will not increase. Therefore, pressure sensing pneumatic solenoids were installed in the contaminant feed lines to the domes. These solenoids monitor the pressure output of the flow transmitters and vent the contaminant feed immediately if the dome flow decreases below a preset level. With this arrangement, high contaminant concentrations are prevented under an emergency condition. In addition, the physical location of the solenoids was changed to the immediate area of contaminant generation. This eliminates the buildup of contaminant in long feed lines when they are connected to the atmospheric vent.

2. Control Line Modification

All control lines in the Altitude Facility were previously installed with polyethylene tubing. Continual problems were encountered with accidental rupturing of these lines in rigid metal conduit up to the general area of usage. The polyethylene lines were terminated in metal junction boxes from whence 1/4-inch copper lines were installed to the appropriate equipment (Figure 2). Accidental rupturing of signal lines is, thereby, reduced to a minimum, and identification and tracing of the system circuitry is enhanced.

3. Ambient Rochester Chamber B

The air supply and exhaust systems to Rochester Chamber B were modified to provide increased flow capability and more precise flow measurements (Figure 3). The orifice plate flow element in the exhaust duct was replaced with a Meriam laminar flow element with a range of 0-100 cfm. Full range output of this unit is 0-4 inches of H_2O differential pressure. This output was supplied to an existing Taylor differential pressure transmitter with an output of 3-15 pounds per square inch. The output of this transmitter was supplied to the existing flow recorder for Chamber B, providing recording and readout on the control panel of 0-100 cfm chamber air flow. Since the original

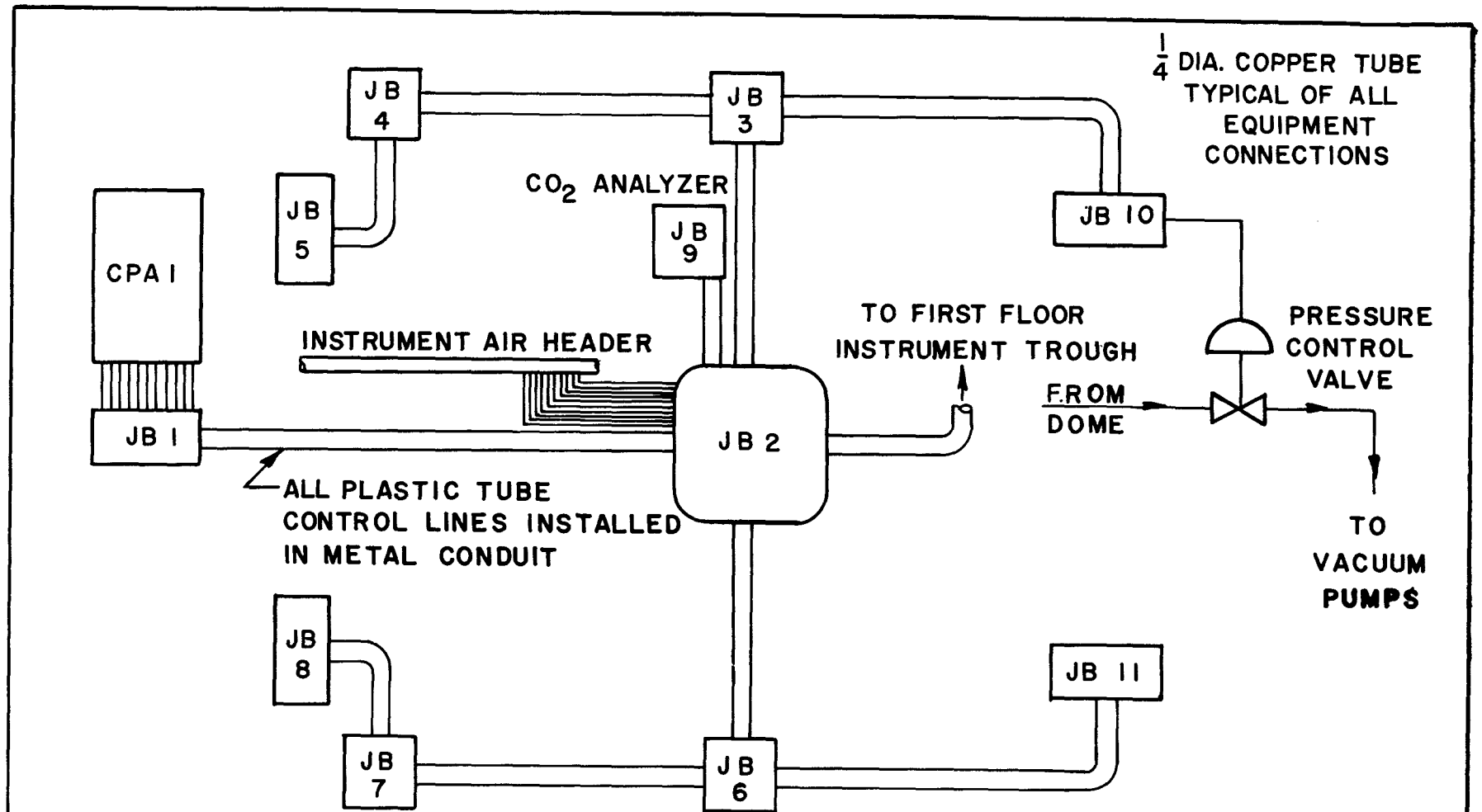


FIGURE 2

ALTITUDE FACILITY CONTROL LINE MODIFICATION

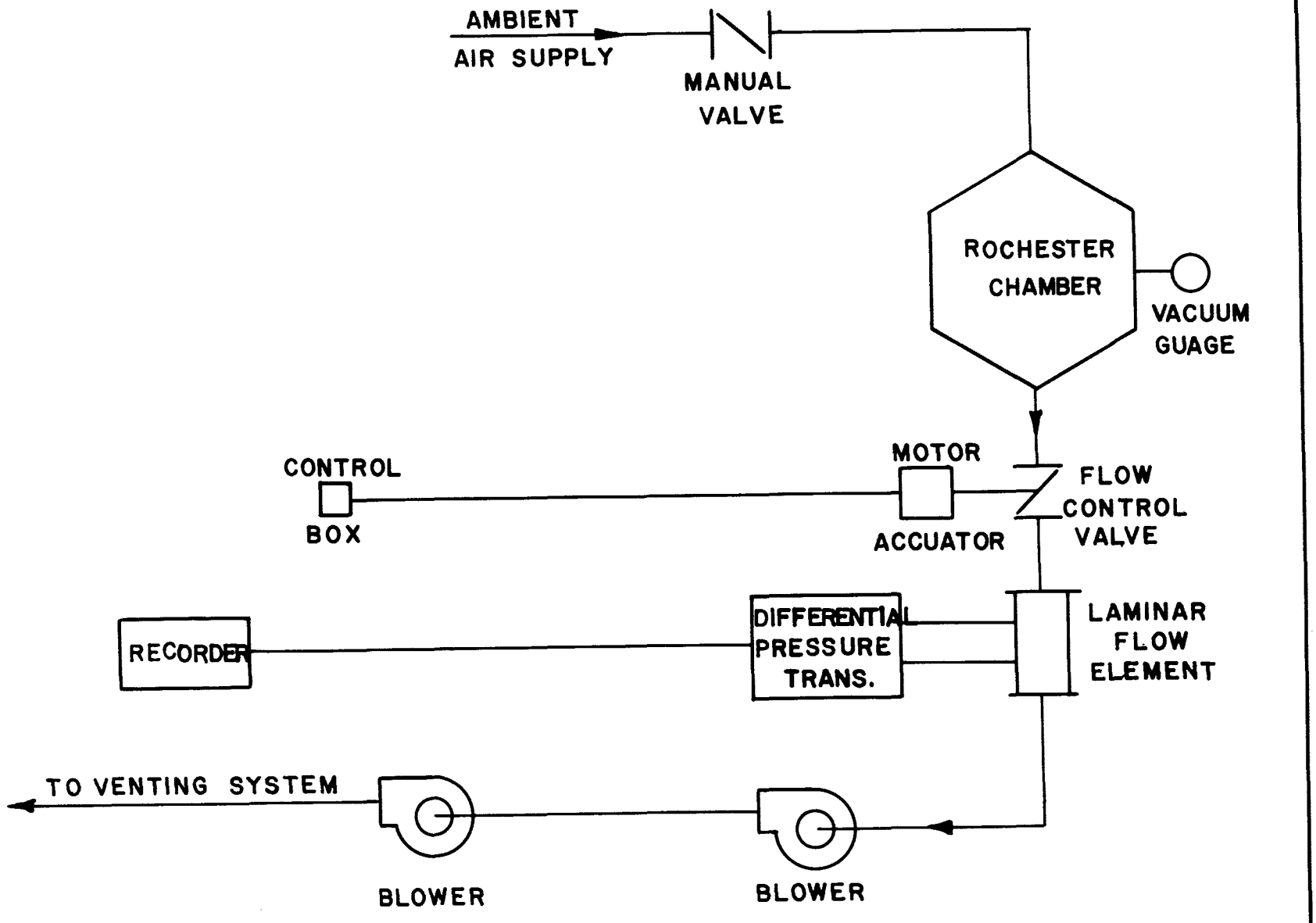


FIGURE 3

ROCHESTER CHAMBER B MODIFICATION

exhaust blowers for the Rochester Chamber did not provide sufficient pressure and flow, they were replaced with a high flow-and-pressure pump with a capacity exceeding 100 cfm flow and six to eight inches of H_2O suction pressure. In addition, a butterfly valve with electric actuator was installed in the chamber exhaust line to provide for remote adjustment of chamber air flow at the control panel. These modifications have proved so successful that future plans call for the same to be performed on Rochester Chamber A.

4. Manual Shutoff Valves in Dome Flow Line

As proposed in the 1966 annual report (Reference 8), manual butterfly valves have been installed in the dome input flow lines between the flow control valves and the entrance into the dome (Figure 4). In an emergency and with the domes at altitude, emergency repairs may be accomplished without a subsequent disturbance to any experiments in progress.

5. Emergency Lights - Ambient Laboratory

Battery operated emergency lights have been installed at strategic locations in the Ambient Laboratory and Animal Room (Figure 5). This extends the emergency lighting capability during power failures to the total area which night shift personnel normally occupy, insuring safe movements to accomplish emergency procedures.

6. Dome Relative Humidity Control System

Preliminary design criteria have been formulated for the relative humidity control system (Figure 6). Final assembly and checkout of control parameters cannot be completed until resumption of 100% O_2 studies. Final calibration of components must be effected with an oxygen atmosphere comparable to that ordinarily utilized during altitude experiments.

7. Animal Weighing System

A prototype model of the platform balance of the animal weighing system has been completed (Figure 7). Preliminary results have indicated that the completed system will yield excellent accuracy and precision. Problems have been encountered with the digital printer readout. At present, it is at the factory for repairs. Work is scheduled to continue with the assembly of this system with a digital voltmeter for the weight readout; the printer will be added at a later date.

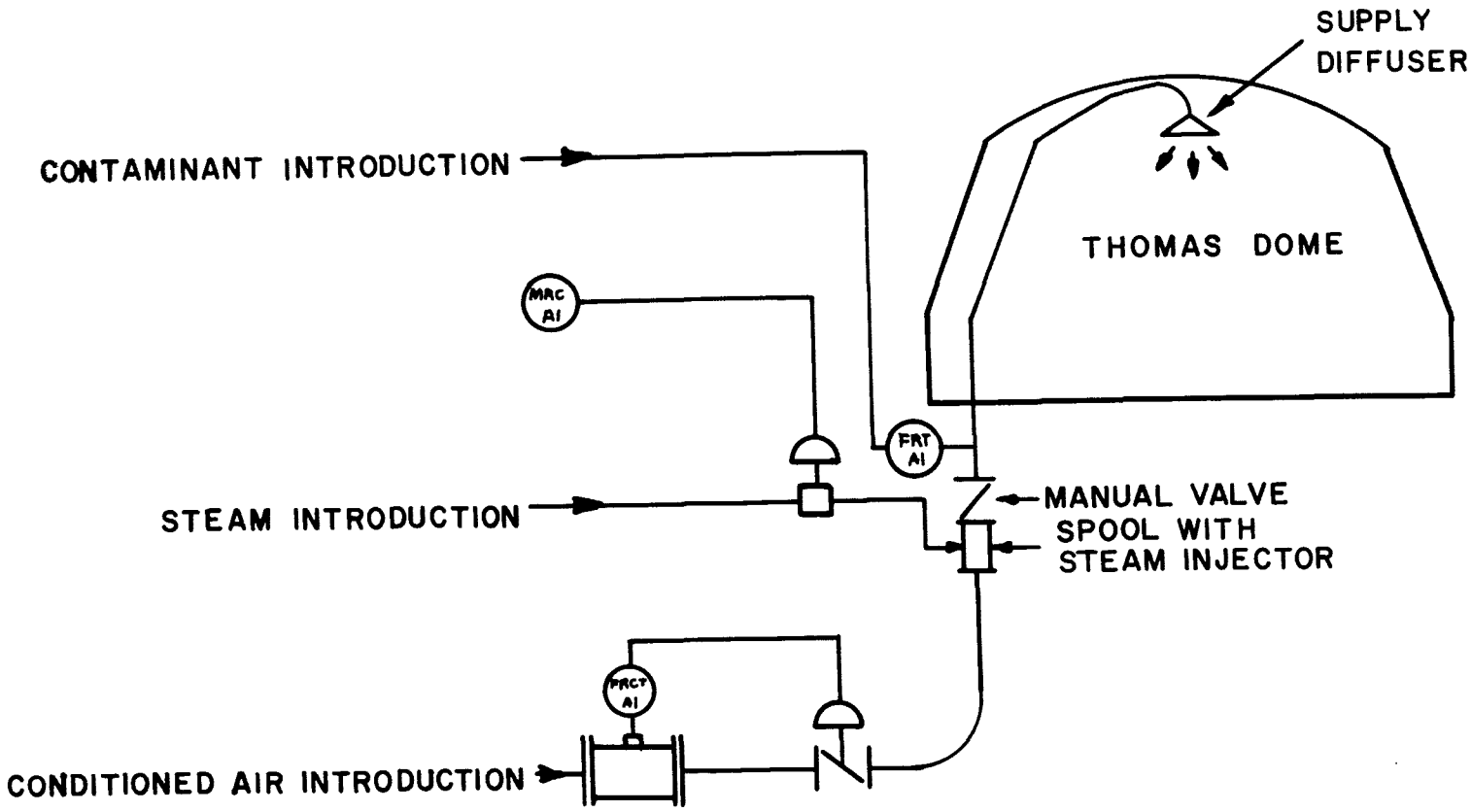


FIGURE 4

THOMAS DOME MANUAL SHUTOFF FLOW VALVE

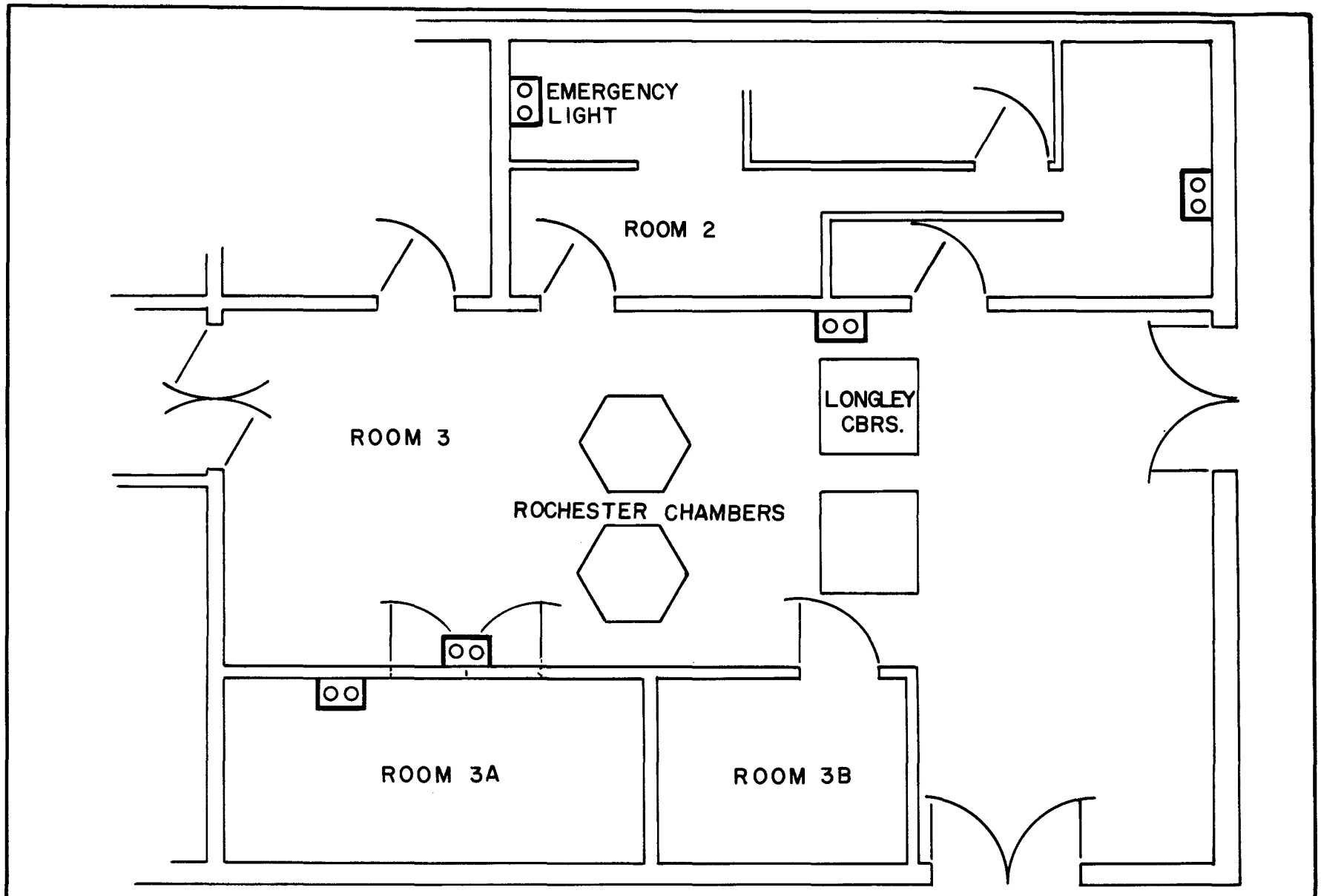


FIGURE 5

EMERGENCY LIGHTS-AMBIENT LABORATORY

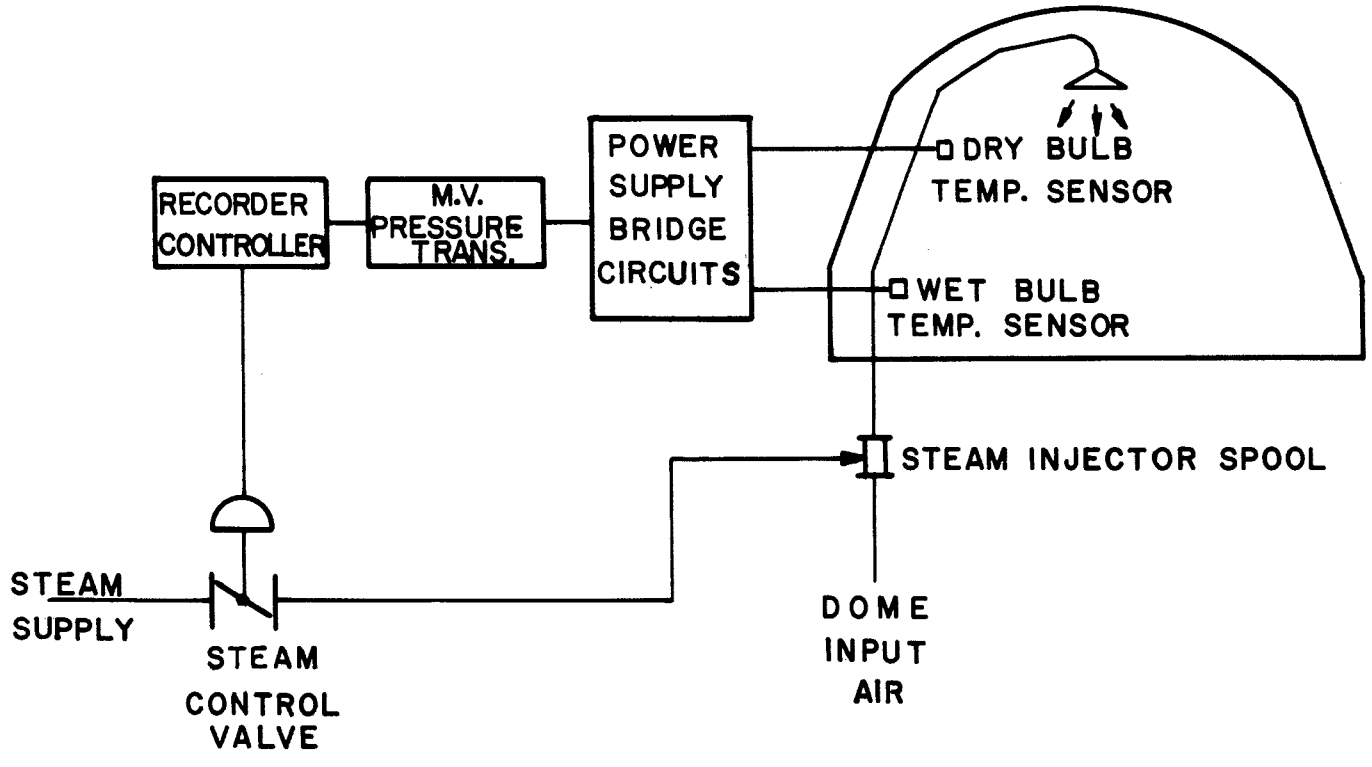


FIGURE 6

THOMAS DOME RELATIVE HUMIDITY CONTROL SYSTEM

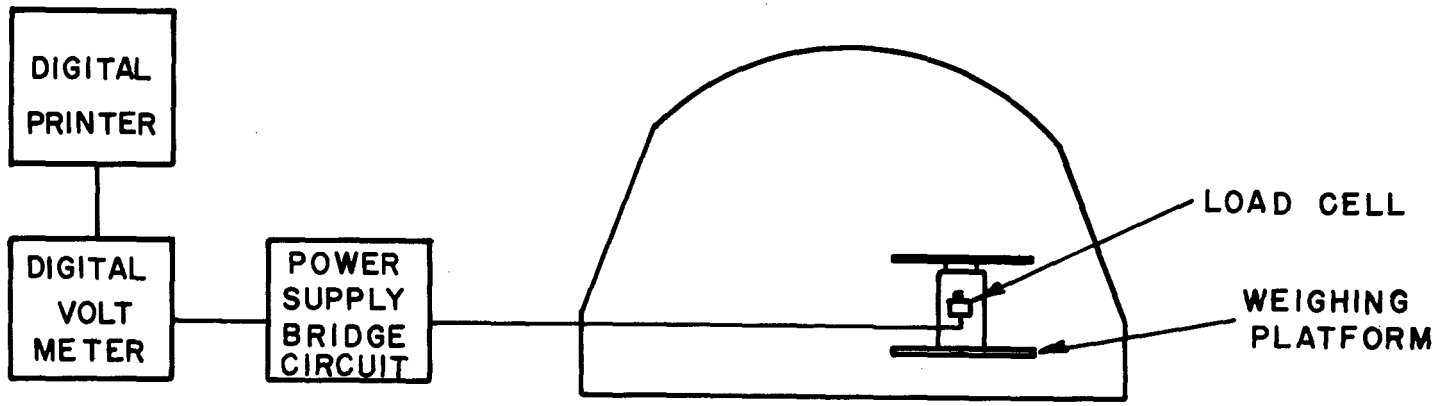


FIGURE 7

ANIMAL WEIGHING SYSTEM

8. Animal Holding Facility

Modification of four large, cylindrical chambers have been completed (Figure 8). These chambers are currently being used to house control animals. Modifications to the chambers included fabrication of stainless steel mesh floors, replacement of Plexiglas windows, strengthening of monkey cages, installation of animal watering systems, and installation of light fixtures in each chamber. Various species of animals can be housed in the chambers for control studies when experiments are in progress. This should be a great improvement over previous arrangements.

9. Automatic Sampling System

Final assembly of the automatic sampling system is in progress (Figure 9). Ninety percent of the needed components have been delivered and are ready for installation. The system as designed is capable of maximum flexibility in sampling contaminant concentration of dome atmospheres. Provision has been made for both continuous and sequential sampling of any combination of domes.

10. Emergency Controls

A system of emergency controls has been installed at the observer stations located adjacent to each Thomas Dome (Figure 10). Controls provided are dome isolation, water deluge, and CO_2 deluge. These controls are pneumatic in operation, with both normal and emergency air supplies provided. Extensive testing at the Aerospace School of Medicine, Brooks Air Force Base, San Antonio, Texas, has resulted in the specifying of a water deluge system in current specifications for fire extinguishment within space cabin and/or chamber simulators. This system shall be automatically activated with a manual override for backup. Consequently, the CO_2 control will no longer be used but will be modified to provide a remotely controlled dump valve. This system could be adjusted to provide any desired rate of descent; whereas in the present system, the rate of descent is difficult (if not impossible) to control. In addition, it would eliminate the necessity of an observer standing on the dome base to activate the present dump valve. All controls can be readily adapted to whatever final design of automatic controls is specified.

11. Dome Penetration Plates

Problems have been continually encountered with the original design of the dome penetration plates. These plates have been modified to provide more easily accessible dome input connection (Figure 11). Permanent

Figure 8. Bldg. 429 (Animal Holding Facility)

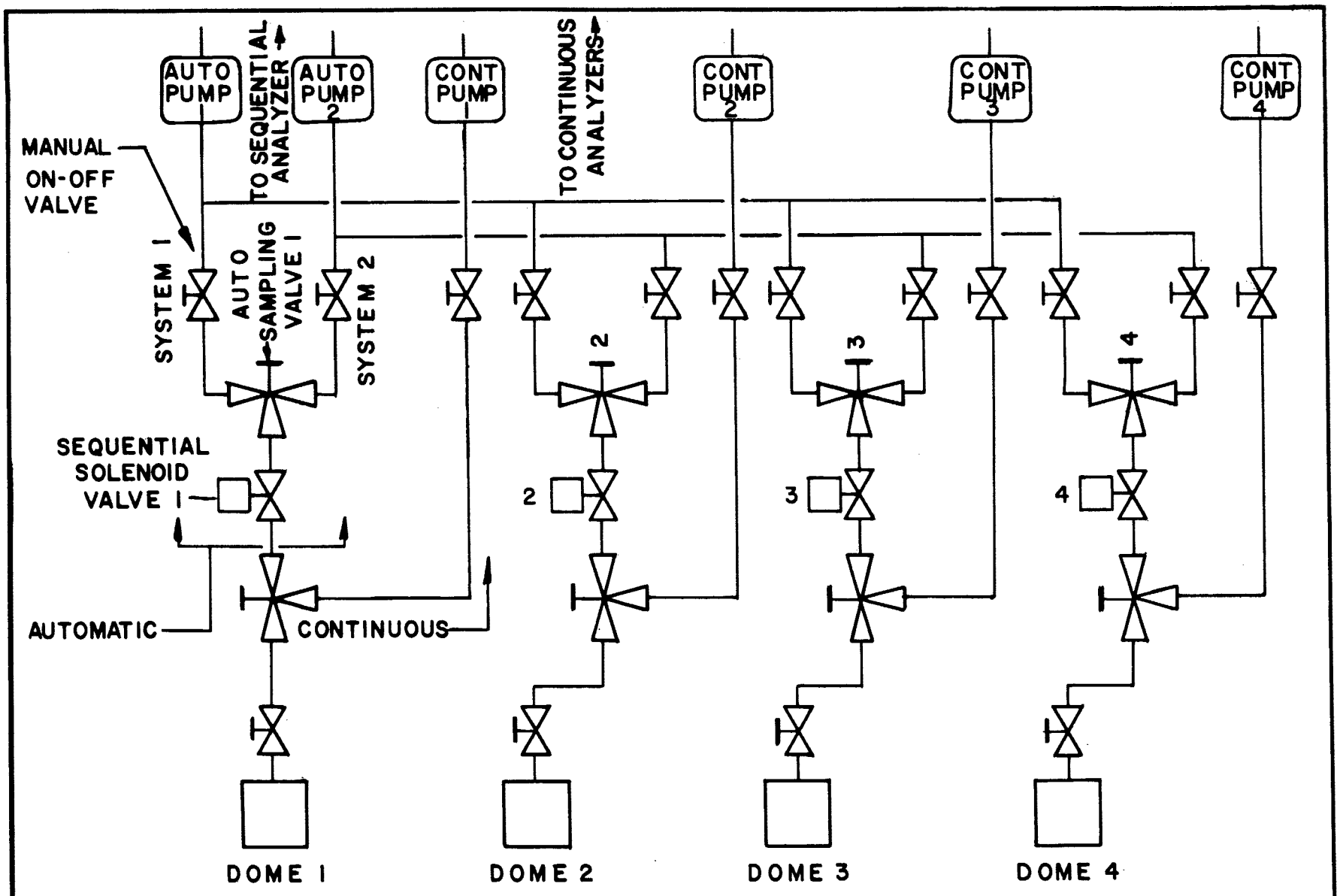


FIGURE 9

AUTOMATIC DOME ATMOSPHERE SAMPLING SYSTEM

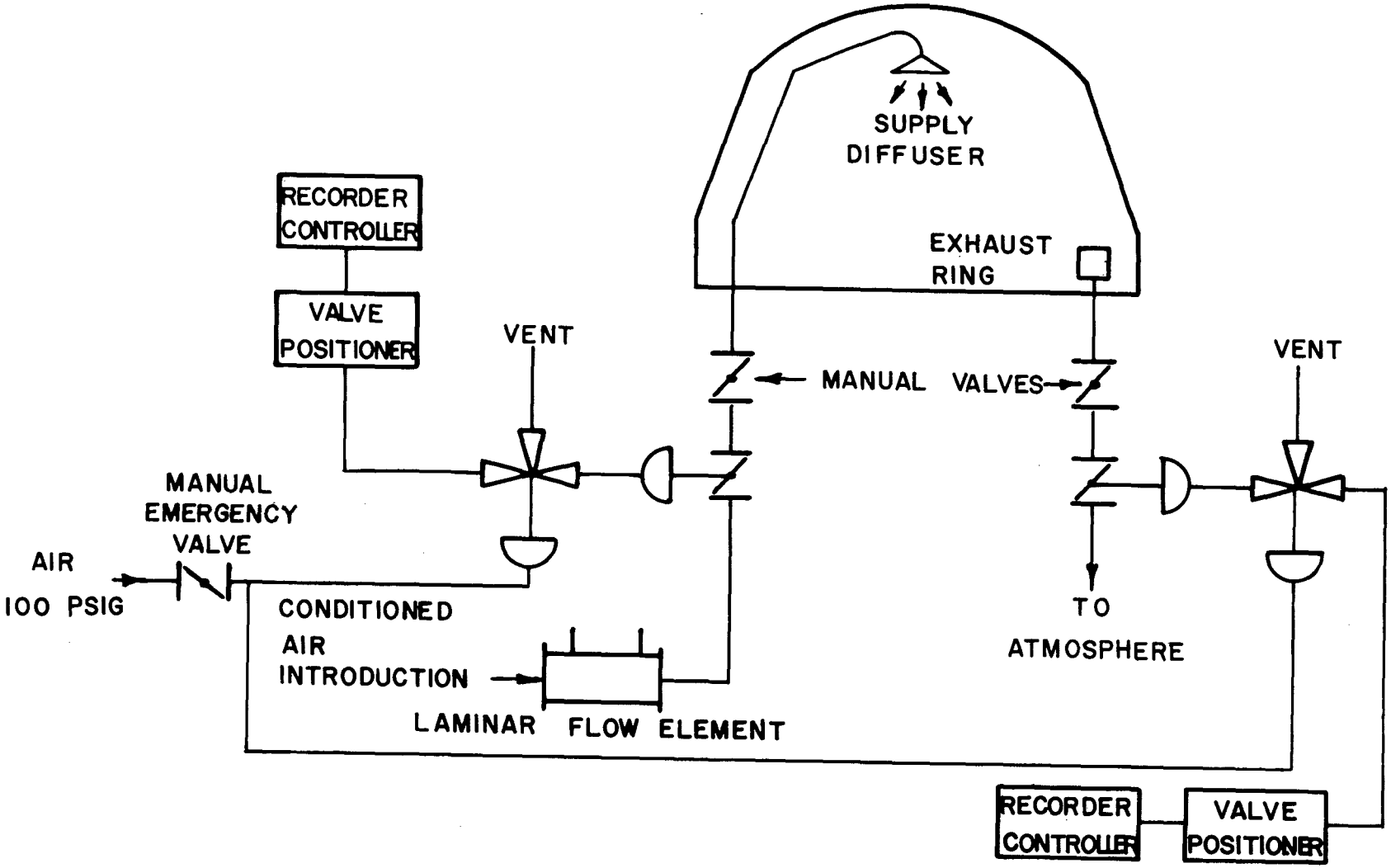


FIGURE 10

DOME EMERGENCY CONTROLS

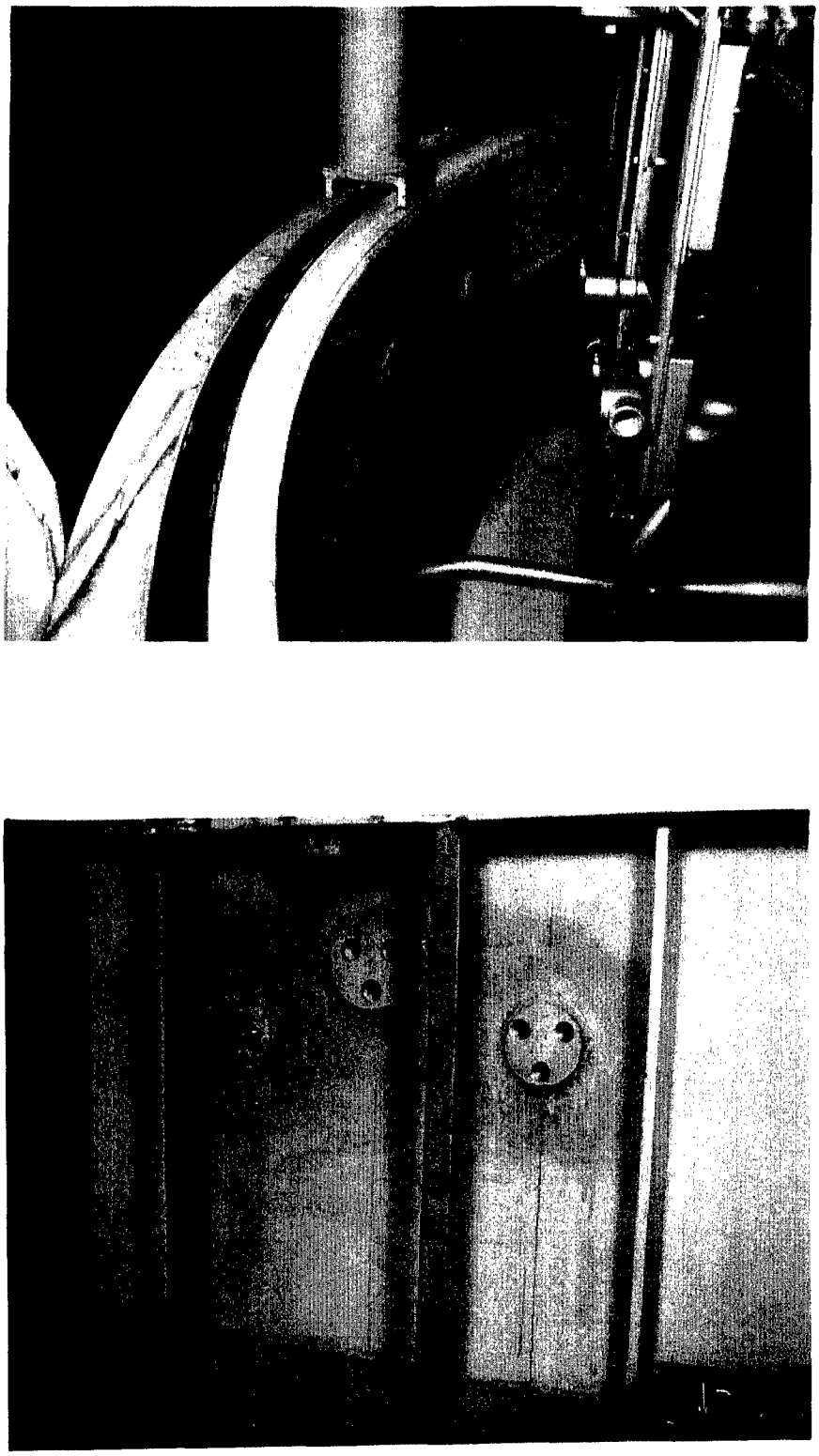


Figure 11. Internal View (Upper) and External View (Lower) of Dome Penetration Plates

connections have been provided to the interior of the dome, with several spare ports available, and all electrical cabling from the penetration plate to the interior of the dome is enclosed.

12. Animal Watering System

The animal watering system for the domes has been modified as shown in Figure 12. The original system included separate regulators and filters for each dome. Maintenance requirements were excessive and entrance into the domes was required for service. The regulators and filters installed in each dome were removed and a single regulator-filter system was installed external to the domes. Thus, preventive maintenance can now be effected without dome entry.

13. Dome Connector Panels

Current safety requirements of 100% O_2 environment chambers require that all permanent wiring shall be teflon (or equivalent insulation) or be enclosed in metal conduit. The open-type signal connector panel rack in the domes was replaced with a moisture-proof NEMA type-12 connector box and panel (Figure 13). The electrical cabling to the signal connectors was routed to this box with rigid metal conduit. This provides the required safety protection of combustible wiring in the chambers.

14. Ambient Facility Venting System

A system was designed to provide venting of hazardous gases and fumes from the Ambient Facility Laboratory to a safe area (Figure 14). A high pressure-high flow blower was installed and connected to an existing outside discharge duct. Four-inch stainless steel tubing was then installed to critical areas of the laboratory. Vent openings are provided for both the Longley and Rochester Chambers and the small hood used for MMH studies. A temporary hookup to this system was made to provide exhaust flow for the Rochester Chamber B modifications previously mentioned.

15. Ambient Air Conditioning System

The results of modifying Rochester Chamber B in the Ambient Facility have shown considerable improvement of atmospheric flow control and measurement. Planning is under way to complete designing for all chambers in the Ambient Laboratory. This system will provide 0-100 cfm flow in each chamber, panel control of flow to each chamber, more efficient control of relative humidity, and 1 percent accuracy in readout of flow values (Figure 15). Input air conditioning components may need to be modified to compensate for the increased flow.

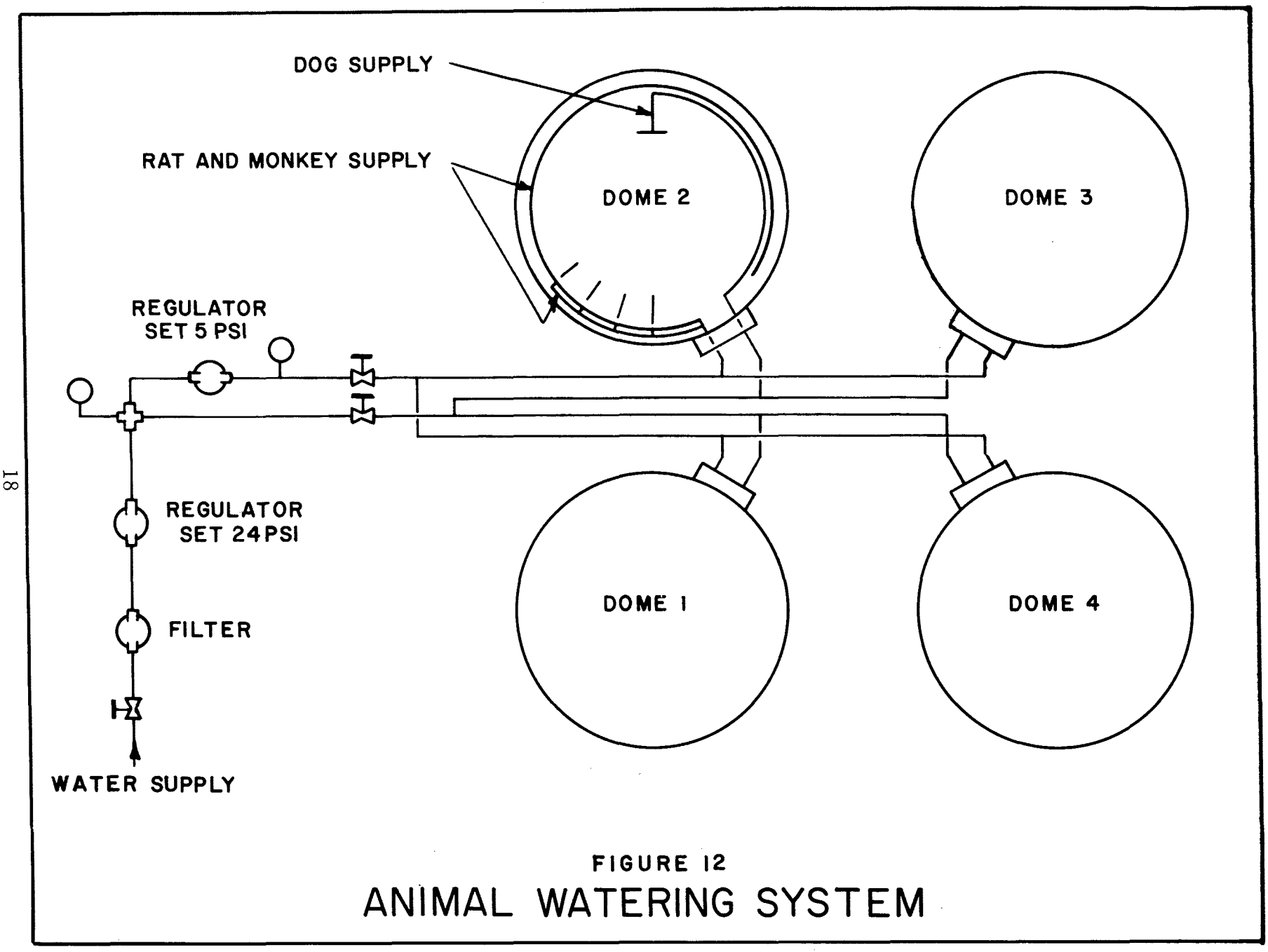


FIGURE 12

ANIMAL WATERING SYSTEM

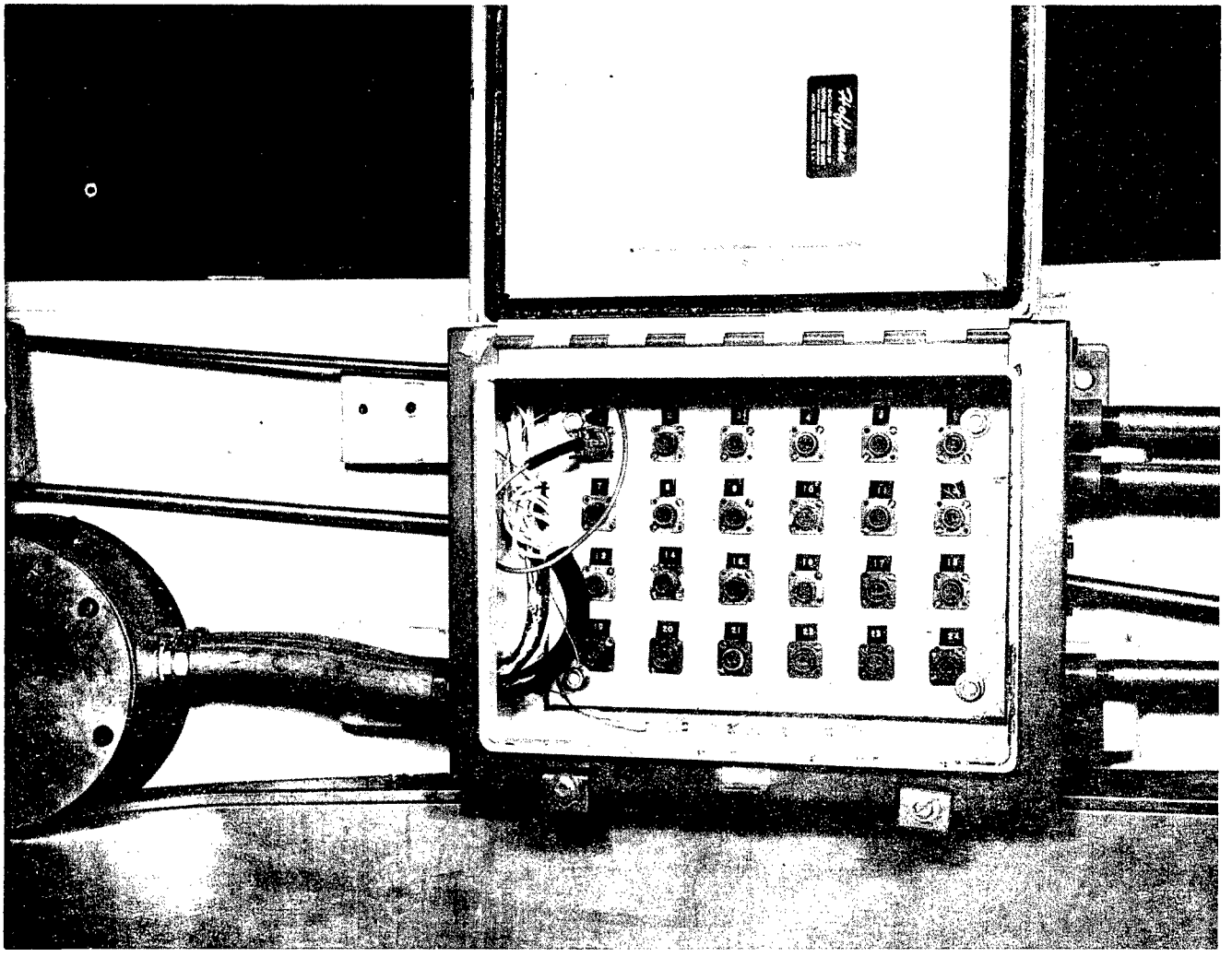


Figure 13. Dome Signal Connector Panel

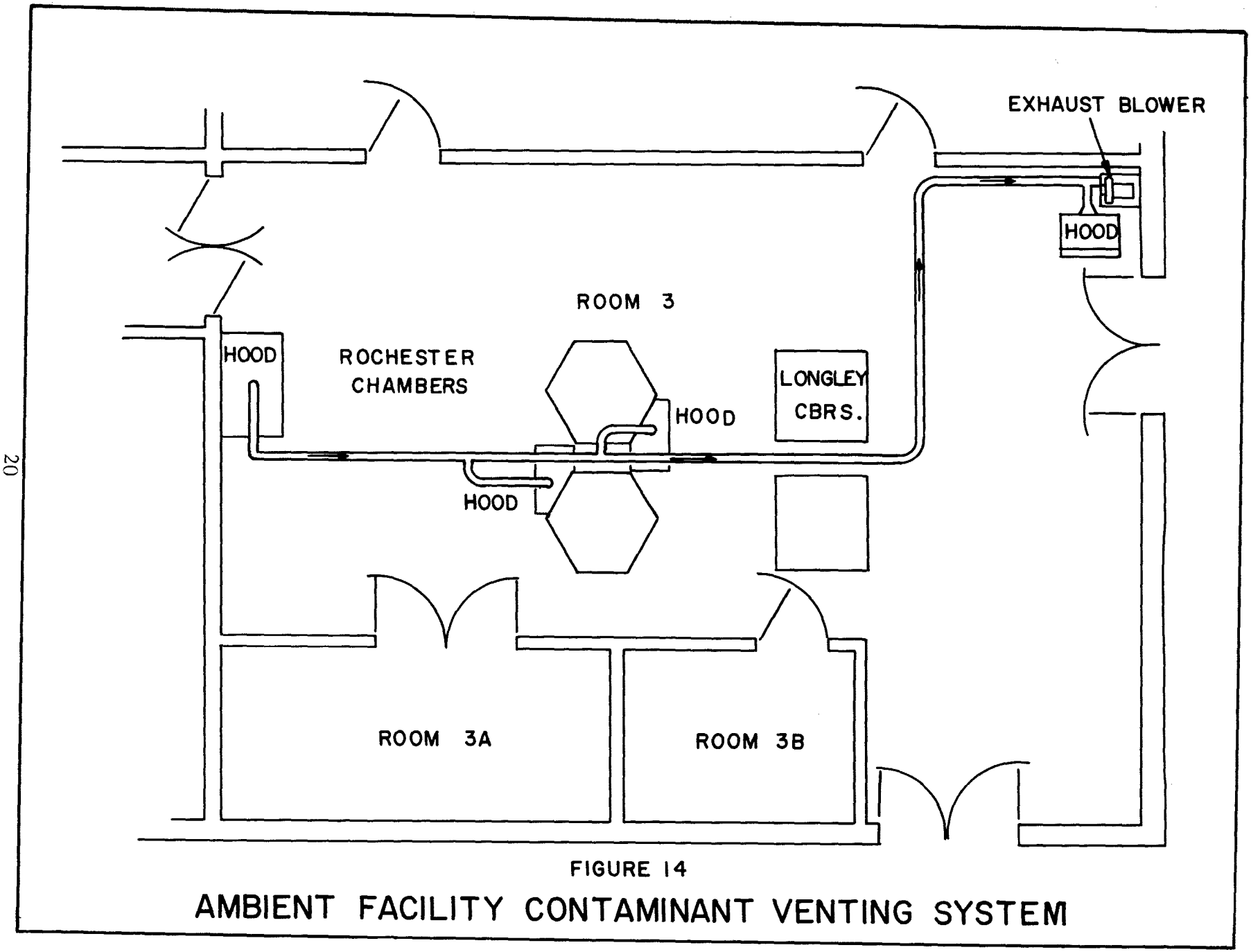


FIGURE 14

AMBIENT FACILITY CONTAMINANT VENTING SYSTEM

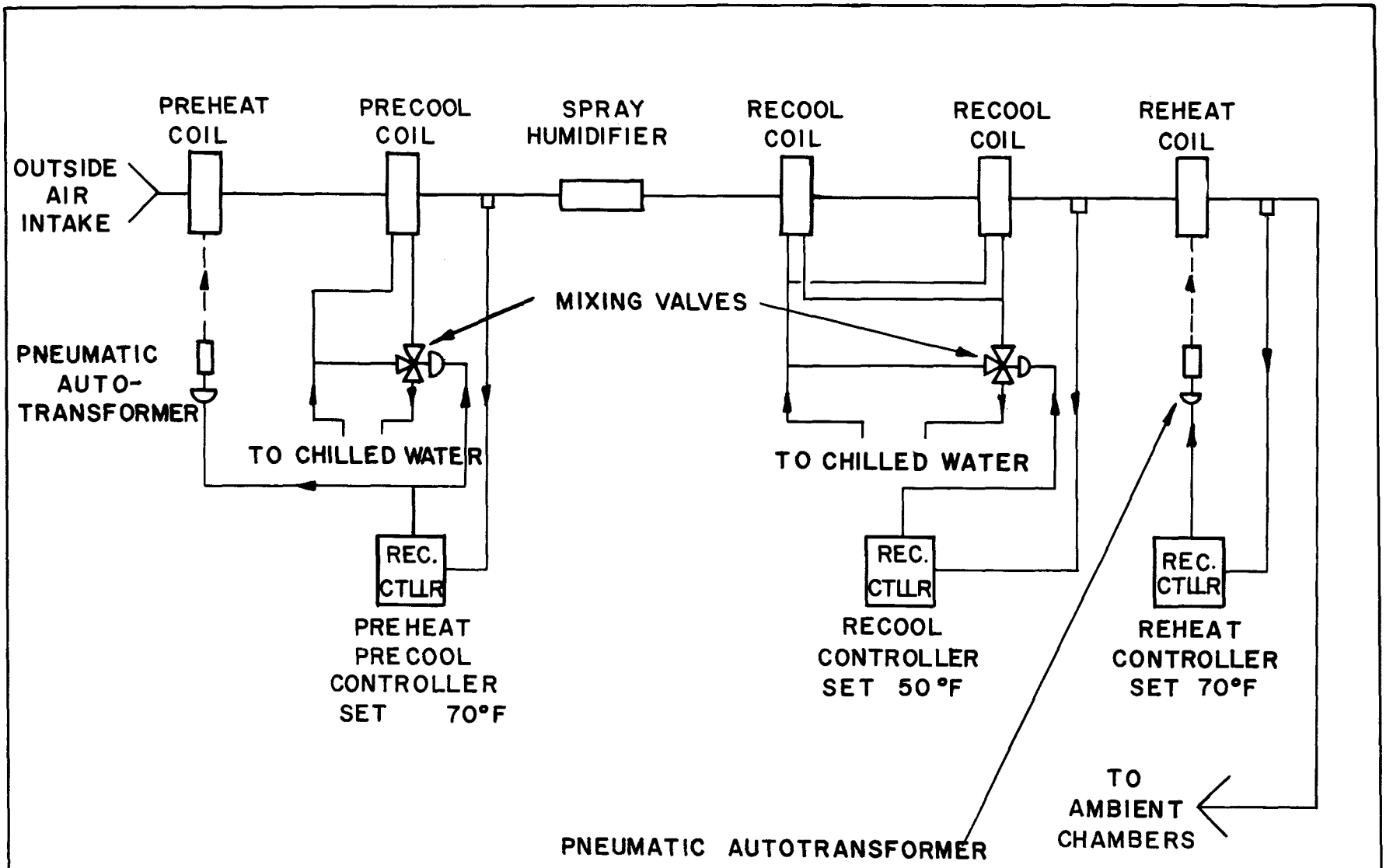


FIGURE 15

AMBIENT FACILITY AIR CONDITIONING SYSTEM

16. Manned Orbital Laboratory (MOL) Systems

The modification of Thomas Dome 1 to accommodate a closed-loop life support system similar to the Apollo space cabin materials testing system in Dome 2 is well under way. Preliminary designs have been completed and fabrication is proceeding in certain areas. Several component sections of the system have been combined because of space requirements. Major components of the system are:

a. Chamber Environmental Support Console (3 each)
b. Atmospheric Monitoring and Control Panel
c. Atmospheric Sampling Rack
d. Thomas Dome 1

There are several significant design changes between the Apollo system and the MOL system. The Apollo system contained three animal chambers supplied by a combined life support console. In the MOL system, the life support system components have been combined on a common rack with their respective animal chambers (Figure 16). This simplifies considerably the plumbing and assembly of units in the dome. It also improves the routine operating procedures required of the chamber personnel. Removable cages have been designed which will permit more efficient handling of animals. Spare cages will be constructed so that replacement of a complete cage may be made if necessary (Figure 17). Service wiring and plumbing are to be installed in the base of the dome, eliminating flexible connections required for a penetration plate in the dome cap (Figure 18). CO_2 and O_2 concentration monitoring and recording and chamber environmental support console control and monitoring have been combined in a single three-section rack panel (Figure 19). Oven temperature is now monitored and controlled from a controller on the front of the panel (Figure 20). Another controller provides for adjustment of diluent concentration during experiments using oxygen-helium gas mixtures (Figure 21). Recording functions have been combined in a single twelve-point recorder monitoring wet-dry bulb temperatures and CO_2-O_2 concentrations for each life support system (Figure 22).

B. CHEMISTRY FACILITY

Special emphasis was placed during the past year on continuous analysis of contaminants introduced into exposure chambers. Experience had shown that batch analysis was not sufficient to demonstrate contaminant concentration changes quickly enough for optimum control. Also, it was necessary in acute exposure experiments to be able to see transient concentration changes which could have profound effects on the response of exposed animals. Batch analysis was completely unable to do this. Therefore, with a view toward strengthening

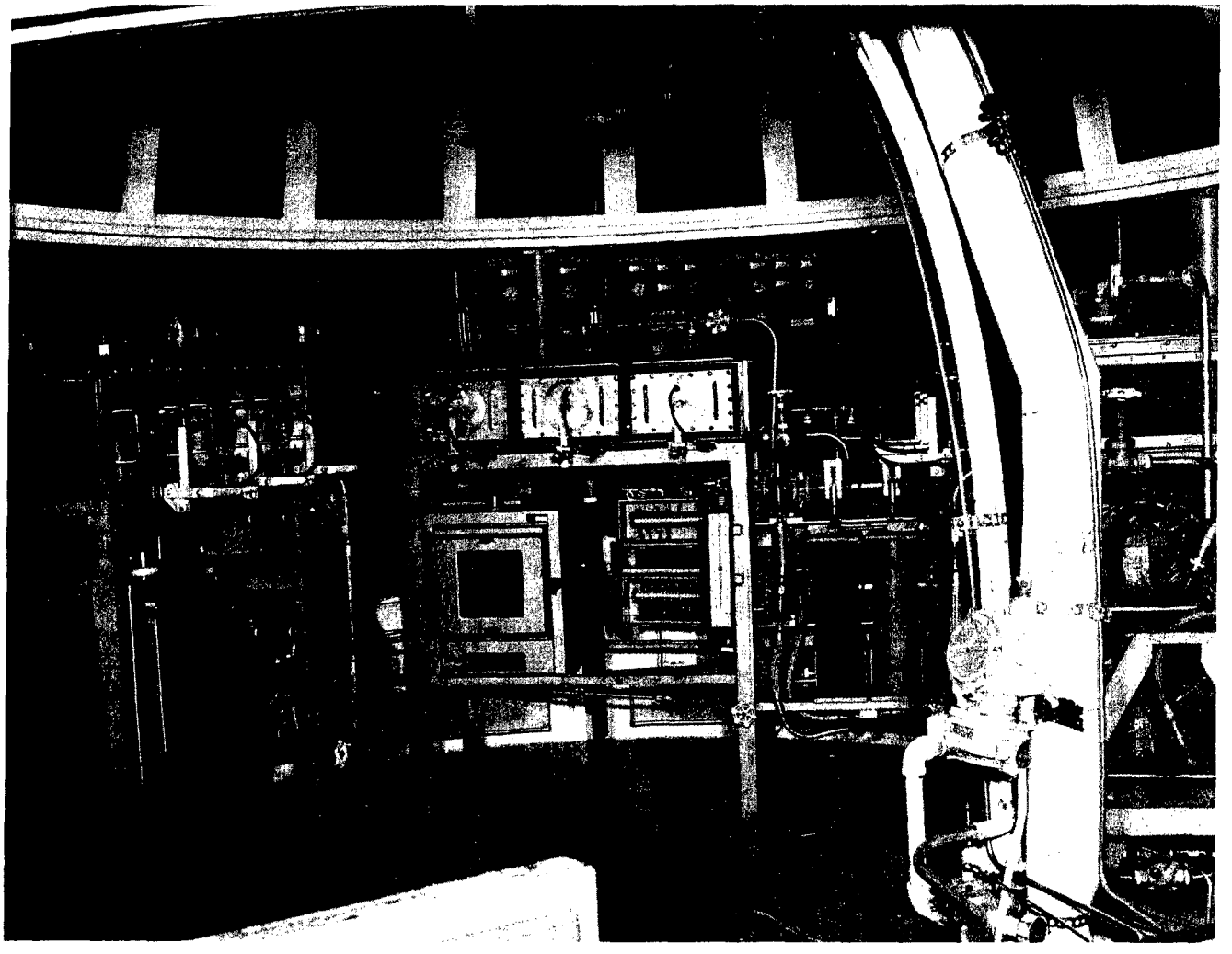


Figure 16. MOL Environmental Support Console

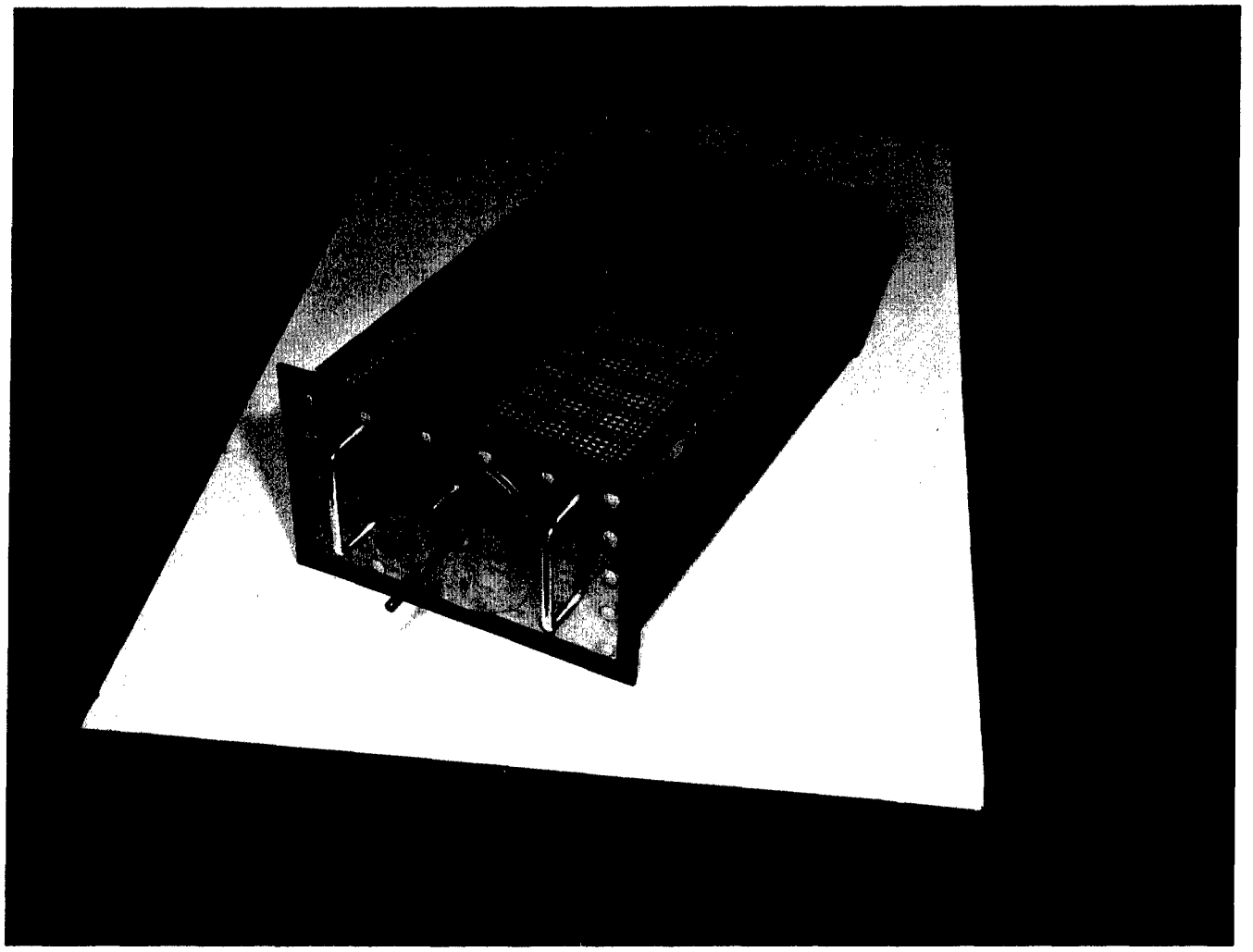
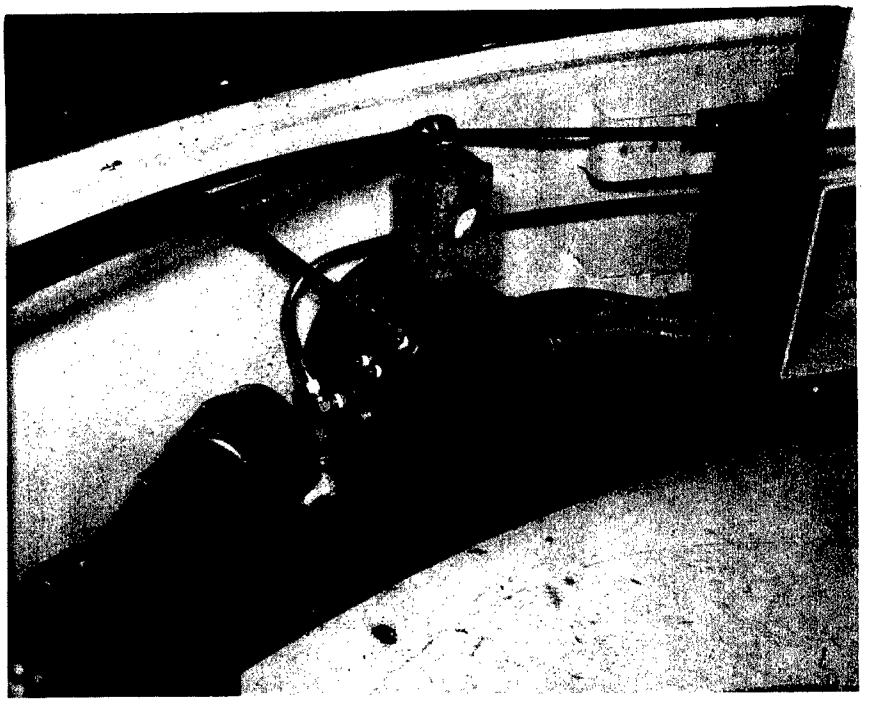


Figure 17. MOL Animal Cage



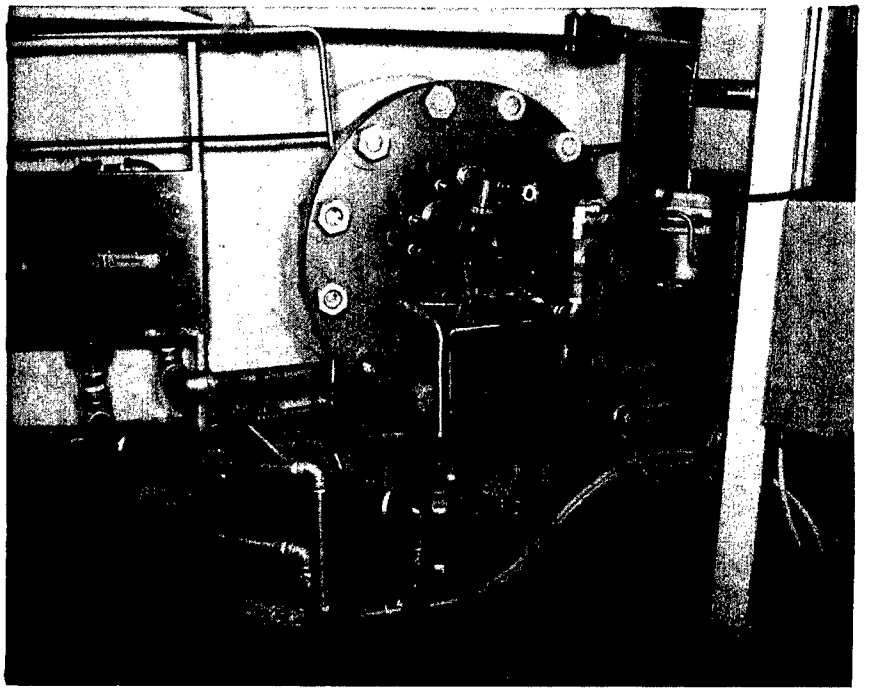


Figure 18. Internal View (Upper) and External View (Lower) of MOL Dome Penetration Plates

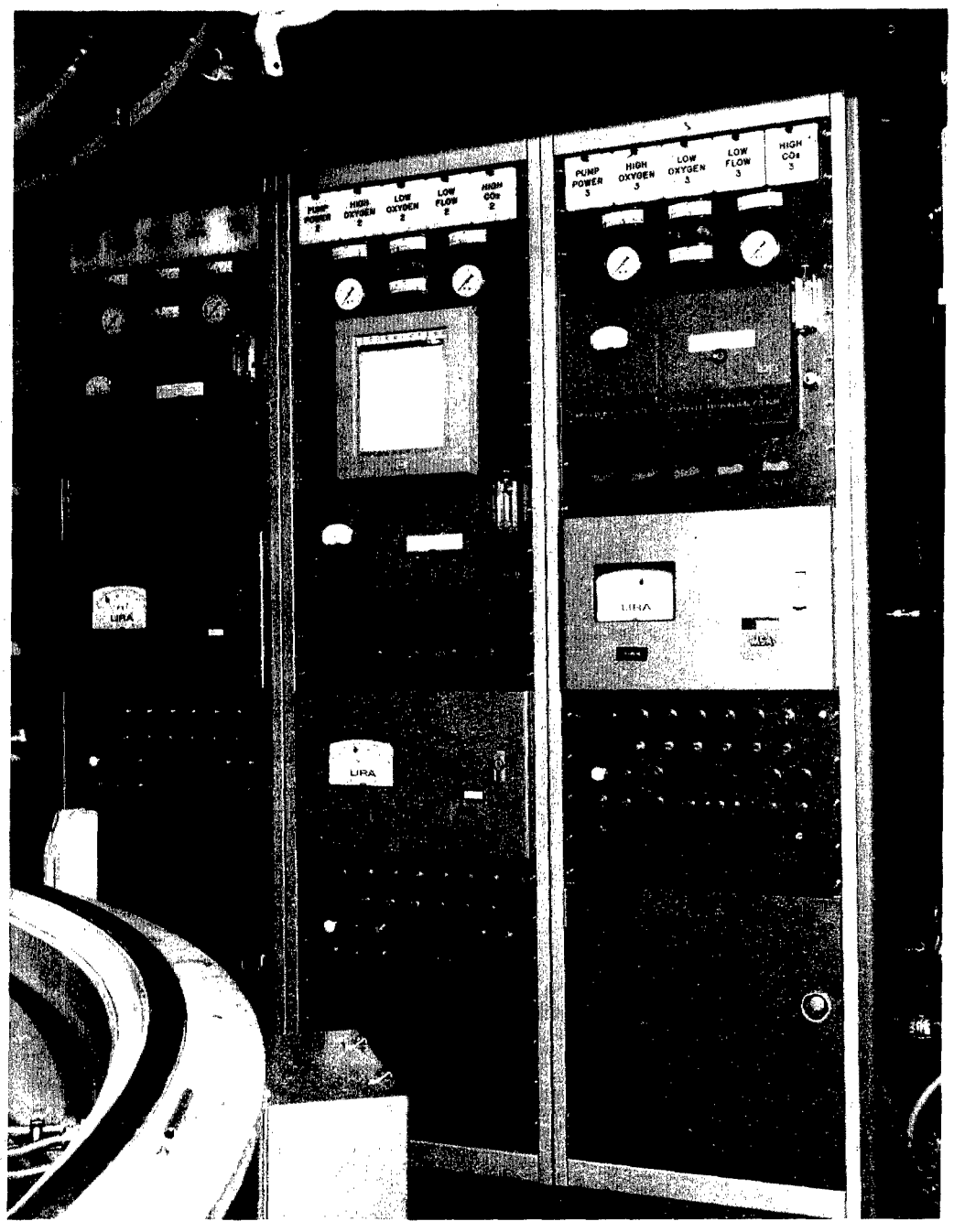


Figure 19. MOL Monitoring and Control Panel

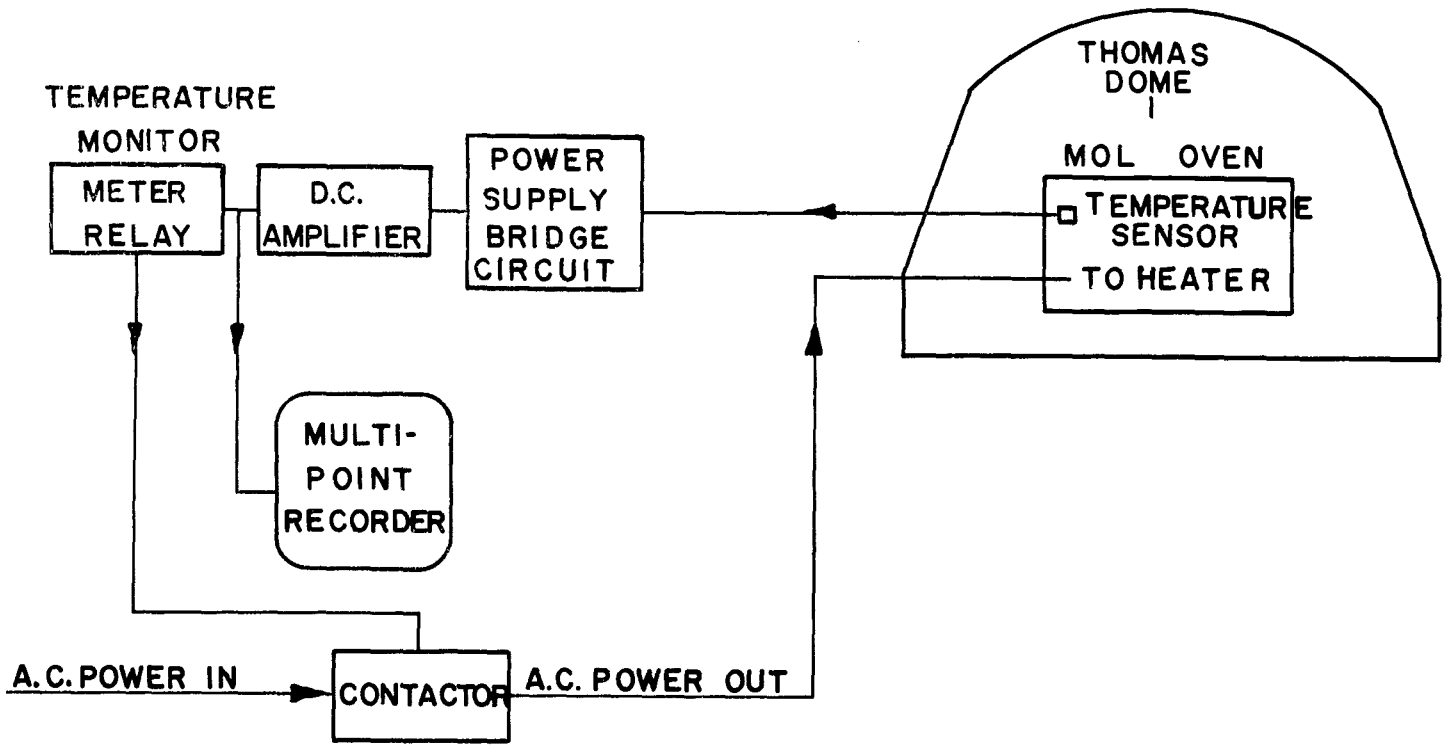


FIGURE 20

MOL TEMPERATURE CONTROL SYSTEM

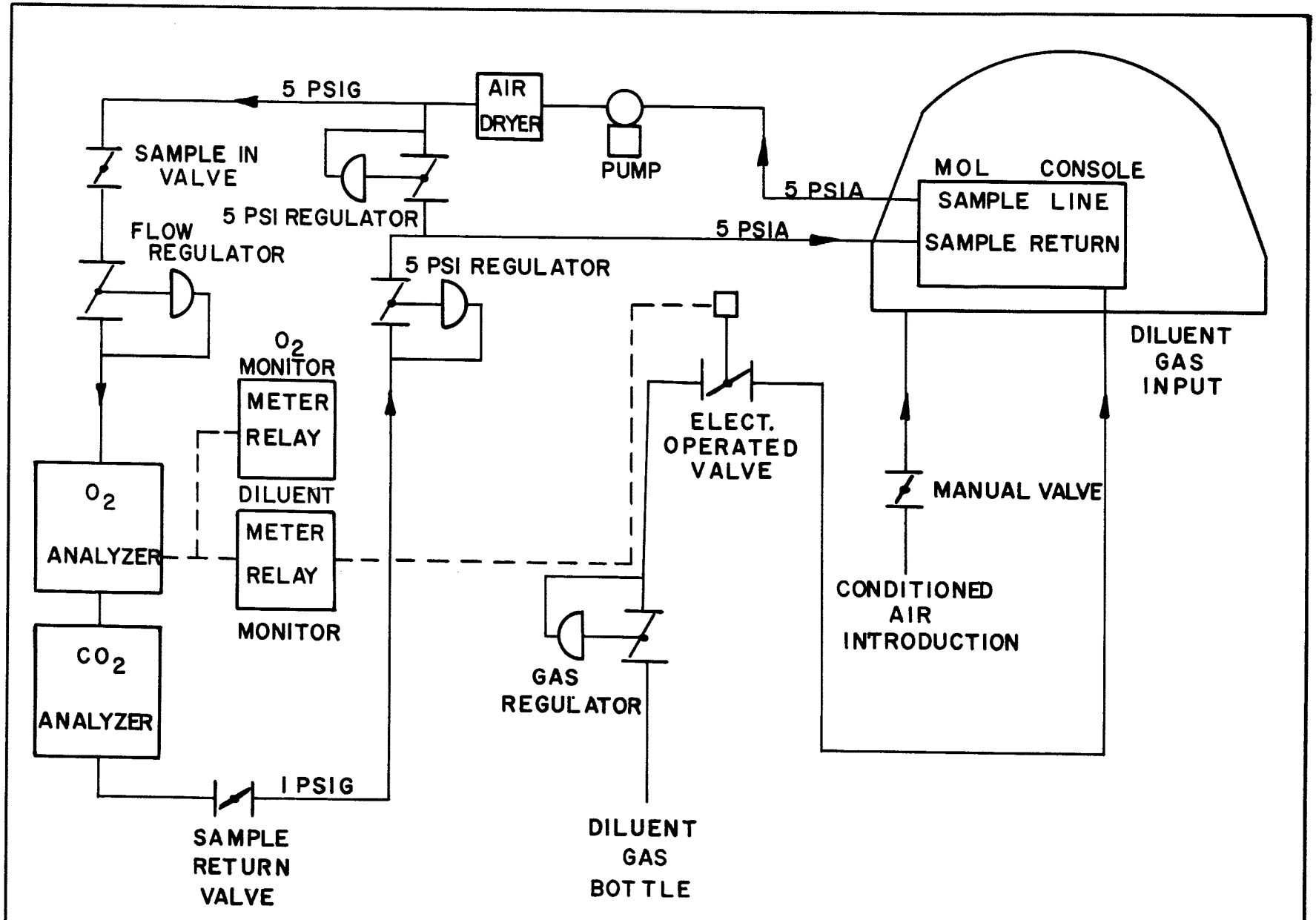


FIGURE 21

MOL DILUENT GAS CONTROL SYSTEM

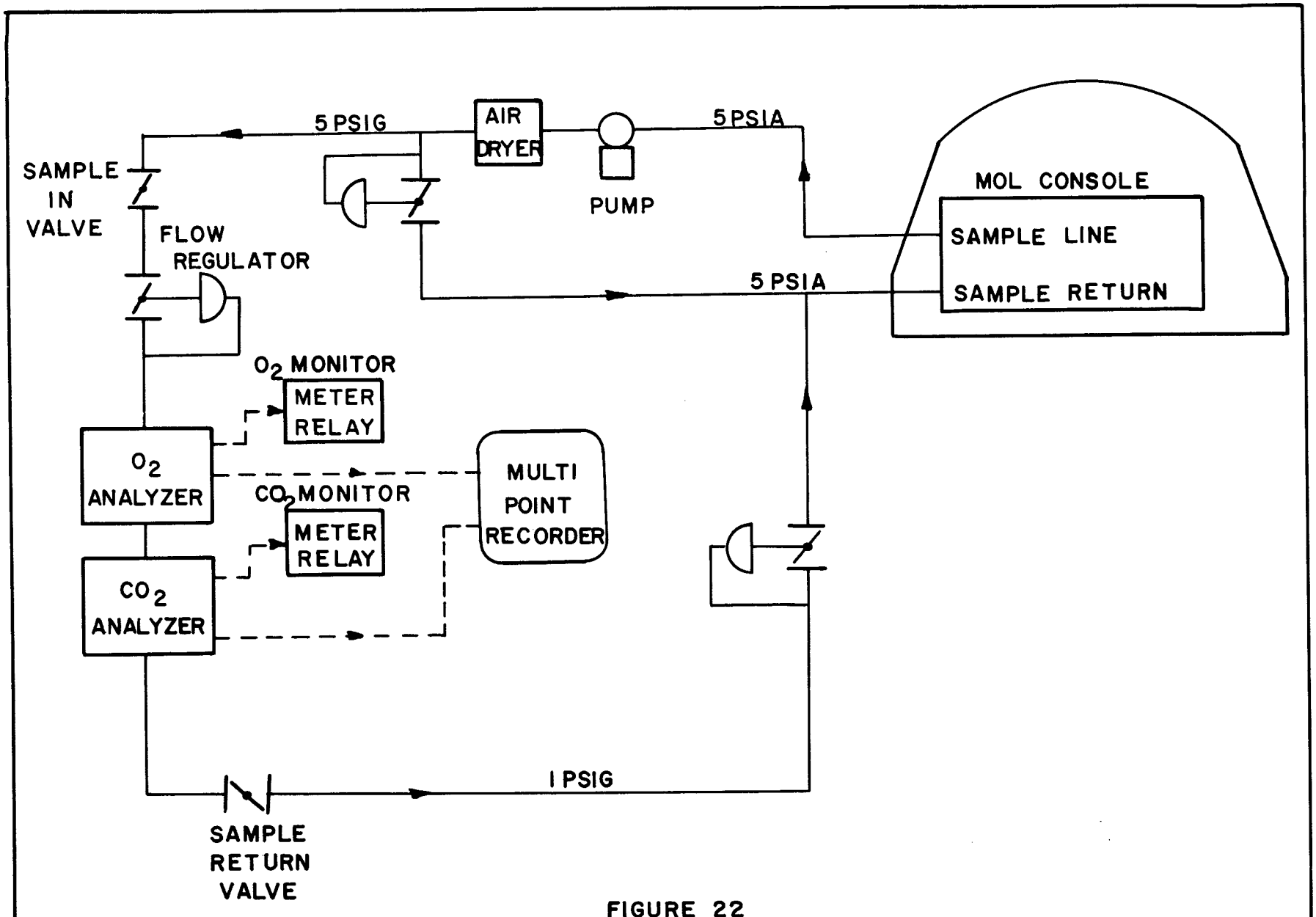


FIGURE 22

MOL CO_2-O_2 RECORDING SYSTEM

THRU's continuous analysis capability during the past year, a number of instruments were purchased. These included three Mine Safety Appliance Billion-Aire Analyzers and two Technicon Auto-Analyzers. A Mast Ozone Analyzer was also procured, making a total of two of these. Utilization of these instruments has been as follows:

1. A method was developed and put to use for the continuous analysis of high NO_2 concentrations (70 mg/M^3) in a 5 psia environment using the Auto-Analyzer.

2. The Mast Ozone Meter was used successfully to monitor Threshold Limit Value (TLV) levels (9.4 mg/M^3) of NO_2 in a 5 psia chamber.

3. Both the Auto-Analyzer and the Billion-Aire were applied to the problem of continuous analysis of monomethylhydrazine (MMH) in acute exposure studies. The Auto-Analyzer accurately measured concentrations of known MMH bag samples which were then used to calibrate the Billion-Aire. The Billion-Aire has been utilized since then to measure MMH exposure concentrations over as short an interval as 15 minutes.

In addition to control analytical instrumentation, a number of research instruments were added to the facility, including a Bendix Polarotrace Polarograph, which can give a complete polarographic curve in seven seconds, and the Canalco Coulometer used for the electroanalytical determination of many materials. An attempt was made to characterize MMH polarographically, but its inherent instability in solution and the press of other work forced a discontinuation of this investigation. A Wilks multiple internal reflection unit was obtained for use with the Beckman Infrared Spectrophotometer IR5A. This has proved extremely useful in the study of opaque or other materials whose physical or chemical characteristics do not lend themselves to use in a solution cell, mull, or KBr pellet. Purchase of an Orion Calcium Ion Activity electrode has permitted the routine daily measurement of water softness using a pH meter and, as a corollary, the efficiency of the water softening unit in the clinical chemistry laboratory.

The Bendix Time-of-Flight Mass Spectrometer has been brought to optimum operating condition. THRU chemistry personnel have attended the Bendix TOF School and have become expert in the operation, maintenance, and repair of the instrument. It is presently being used to investigate the complex mechanism of MMH oxidation and to analyze any unknown compound or mixture which is capable of being vaporized in the instrument. Two Keithley solid state amplifiers were substituted for the previously used IFI oscilloscope amplifier and gave greatly improved noise and stability characteristics.

The two 10-meter gas cells available for use with the IR5A Beckman Infrared Spectrophotometer were disassembled, cleaned, and

reassembled. Experiments which were conducted using $CBrF_3$ as a test gas showed that all the available path lengths from 10 centimeters to 10 meters were giving correct absorbances.

Two systems were designed and constructed for the introduction of ethylene glycol into the Thomas Domes at saturated vapor pressure concentrations (254 mg/M^3). This study is described in a later section. Syringe feeders delivered the required quantity of liquid glycol into a generating flask kept hot by a heating mantle and resistance wire. Room air pulled in by a slight vacuum in the system picked up the glycol vapors and carried them through heated lines and a pressure dropping valve to the dome. Gas chromatographic and colorimetric methods of ethylene glycol were developed which permitted the maintenance of a stable glycol level throughout the exposure.

A small chamber was adapted to the study of the combustibility of materials in 100% oxygen. The chamber can be evacuated to 5 psia and materials can be subjected to 800°C resistance wire temperature under static or dynamic conditions. Many materials suggested for use in 100% oxygen were tested for ignitability, using this apparatus.

Looking forward to Ambient Facility acute exposure studies of oxidizers in the future, a small manifold was constructed to permit the dilution and isolation of samples of oxidizers such as NF_3, N_2F_4, and ClF_3. This arrangement will facilitate development of batch and continuous methods of analysis for these materials and allow for safe handling procedures.

C. COMPUTER PROGRAM SERVICE

In the past year, utilization of the computer facilities at the Data Processing Center has increased. THRU required the analysis of data generated by the TLV experiments mentioned in the last annual report. Accordingly, liaison was established between pertinent personnel, and an appropriate program was selected to produce the necessary analytical manipulations. After numerous mechanical program errors were eliminated, results were predictable. The "F test" was applied to all parameters in a series of hypothesis related to the influence of sex, time of experiment, and variation throughout the course of the experiment.

Presently, data on blood measurements (Table III) from all monkeys and dogs utilized as controls or currently housed at the Vivarium are being prepared in an attempt to clarify the "normal" or "control" limits for blood values of these two species. The means and standard deviations will be computed with the aid of the BMDOID and BMDOIV programs. Effort is being expended on the necessary intermediate sub-routine programs and the processing of the data from raw notebook source to suitable input sheets.

Future plans include the comparison of the long-term oxygen toxicity experiments, i.e., the 8-month 100% oxygen study, the 8-month mixed gas study, and the currently planned repeat of the latter study.

Finally, a survey of organ-weight data gleaned from the numerous Apollo studies, as well as the MOL studies planned for the future, will be attempted. It is hoped that some mathematical model or concept can be developed to aid in the evaluation of this type of data; otherwise, the reams of data collected from a series of studies render the evaluation of "effect" or "no effect" response extremely burdensome, as well as time-consuming.

D. ANIMAL HOUSING

As mentioned herein under Engineering, work was completed on the new animal holding facility. Standard operating procedures were drawn up, and two independent alarm systems were incorporated into the overall warning system; one alarm system, utilizing the present panel board, is activated by a loss in chamber flow and/or a temperature variance from a preset range value; the second alarm system distinguishes between the above operational factors via sound and light signals. The latter system is located in the Ambient Facility, and a third is planned for location in the Altitude Facility. Thus, even though the buildings are physically separated, there will be excellent monitoring of the environmental condition where most of the control animals are to be housed. Operational procedure and signal systems have been checked out with all individuals (technicians, etc.) concerned.

The area formerly designated as the animal room in Building #79 has been renovated and partitioned. Only incoming rodents will be permanently housed in this area once new chronic studies are under way. In this manner, the new rodents can be quarantined in separate housing from those control rodents to be housed in Building #429. Chance for introduction of disease to controls for long-term experiments can thus be minimized.

SECTION III

RESEARCH PROGRAM

GENERAL

Research activities were necessarily limited following the first four months covered by this report. Restrictions placed upon use of the Altitude Facility after the Cape Kennedy Apollo and Brooks Air Force Base fires, which occurred during the month of January, 1967, prohibited the use of atmospheres of oxygen content greater than ambient. Accordingly, the majority of the research phase of THRU's activities since that time has been restricted to: (1) experimental studies with monomethylhydrazine (MMH) in the Ambient Facility, (2) various control studies under ambient conditions in one of the Thomas Domes and in the animal holding facility, and (3) to analytical developments in the chemistry section.

Some of the research conducted in the Altitude Facility and included herein was a continuation of experiments which began prior to the period of this report and are so indicated in Table I. This summarizes all experiments conducted in the Altitude Facility and tabulates the experimental and/or control conditions for each. Similarly, Table II gives account of experiments and conditions for the Ambient Facility during this time.

Preparation for fire tests for assessment of fire extinguishing efficiency of systems in one of the domes has already been alluded to. Inasmuch as this involved certain unknowns, the details of preparation and protocol, as well as outcome, are included under this section on research.

A. ALTITUDE FACILITY EXPERIMENTS

1. Oxygen-Nitrogen Environment at Reduced Pressure

An 8-month study of the effects of a mixed gas environment consisting of 68% oxygen (O_2) and 32% nitrogen (N_2) at a pressure of 5 psia was conducted using Rhesus monkeys, beagle dogs, rats, and mice as experimental subjects. As indicated in Table I, this study and its control counterpart began prior to the period covered by this report and terminated in January, 1967. Certain criteria for assessment of effect were those which have been rather routinely used in study of chronic inhalation exposure to contaminants and/or controlled environments; these are listed in Table III. Additionally, necropsy data, body weight gain, organ and organ-to-body weight ratios, and histopathologic evaluation were utilized.

The findings concerning the outcome of this study were presented at the 3rd Annual Conference in May, 1967 (Reference 3) and are only briefly reiterated here. Differences found between experimental and control species (Table I, #167 and #168, respectively) were:

(1) a slower mean growth rate of exposed rats, i.e., approximately 15% and 8% behind that of the control males and females, respectively, but without statistical significance,

(2) a slight difference between the rodent group mortalities, again without significance,

(3) an inversion of the albumin/globulin ratios exhibited by exposed dogs, in contrast to controls, at approximately midpoint through the 8-month study. This response appeared to be a function of gradual increase of total serum protein content as judged by salt precipitation determination, and would imply that the globulins were contributory since albumin remained relatively consistent. The latter implication, however, was not completely substantiated by limited studies of serum protein fractionation with disc-electrophoresis.

(4) Necropsy data revealed that the liver/body weight ratios of exposed dogs differed significantly from controls. There was correlation here with liver ultra-structural changes as determined by electron microscopy (Reference 6), but light microscopy did not distinguish between control and exposed dogs (Reference 10).

The few differences noted above did not attain magnitudes considered significant in other animal species tested. One other measurement, however, did show a difference of statistical proportions, this being heart and lung-to-body weight ratios for male rats; the organs were proportionately larger.

The findings permit an assumption that the mixed gas atmosphere of 68% O_2 and 32% N_2 was not totally without effect and that some impairment of physiologic function occurred under the conditions of test. Further experimentation involving chronic exposure in a mixed gas environment is indicated.

2. Apollo Materials Toxicity Screening

Rationale and protocol for toxicity screening tests with Apollo space cabin construction materials were briefly mentioned in the last annual report. They are reiterated here for the sake of clarity of this continuing phase of the THRU research programs, and to document the type of screening studies scheduled for the Manned Orbital Laboratory (MOL) construction materials. Just as the basic units for the Apollo and MOL systems mimic each other (see notations under Facilities), so will the testing protocols for each.

Apollo toxicity screening tests were begun in 1965 on behalf of the National Aeronautics and Space Administration. The studies are designed to determine whether gas-off products from cabin construction materials are toxic when presented to animals via inhalation. Rats and mice are exposed to an atmosphere of 100% O_2 at 260 mm Hg pressure containing the gas-off products from materials heated to 155F. Description of the equipment and systems which accomplish this complex task is well documented (References 1, 8). A schematic diagram depicting the current closed-loop life support system is shown in Figure 23. Specific design changes indicating the major differences between the Apollo testing system and those for the MOL studies have already been noted in this report under Section II.

A standard operating procedure has been adopted to determine the quantity of construction materials to be tested, and would apply as well to MOL testing. If the proposed use of the material in the space capsule exceeds 1 pound, a 100-gram sample is tested for gas-off toxicity. If less than 1 pound, a 10-gram sample is used. Basically, any compound meeting the requirements for space cabin construction or equipment, or utilized for interior modifications, may be considered for the screening program. Thus, inks, dyes, resins, plastics, epoxies, and many more generic groupings are included. Approximately 500 candidate materials are scheduled for screening, with about 25% having been completed at the time experiments with high oxygen concentration were suspended.

A series of 7-day screening tests, as well as a 60-day test which was initiated prior to the beginning of this report period, have been concluded. Each of these are indicated by experiment number, duration, and test conditions in Table I. Test protocol, whether experimental (exposure to gas-off products) or control, called for an animal complement of 20 rats and 25 mice in an individual test. Thus, a single experiment could involve five sets of animal complements, these being three for exposure to gas-off products of three different groups of materials (one in each of three life support systems), and two for control purposes. At least one control was always used outside the life support loop, but in the dome environment, and one in the animal room quarters under ambient environmental conditions.

For initial screening purposes, a single experiment involves exposure to gas-off products from a mixture of 18 materials for 7 days. If a definite trend of toxicity is found, e.g., significant loss of body weight, organ damage, etc., the materials are subdivided into smaller groups and retested. Following the 7-day tests, the materials are put in groups comprising 100 each, and these are tested for toxicity resulting from exposure of 60 days duration.

Some tests involved only one or two animal complement groups which received gas-off products, while the remaining groups served as control for

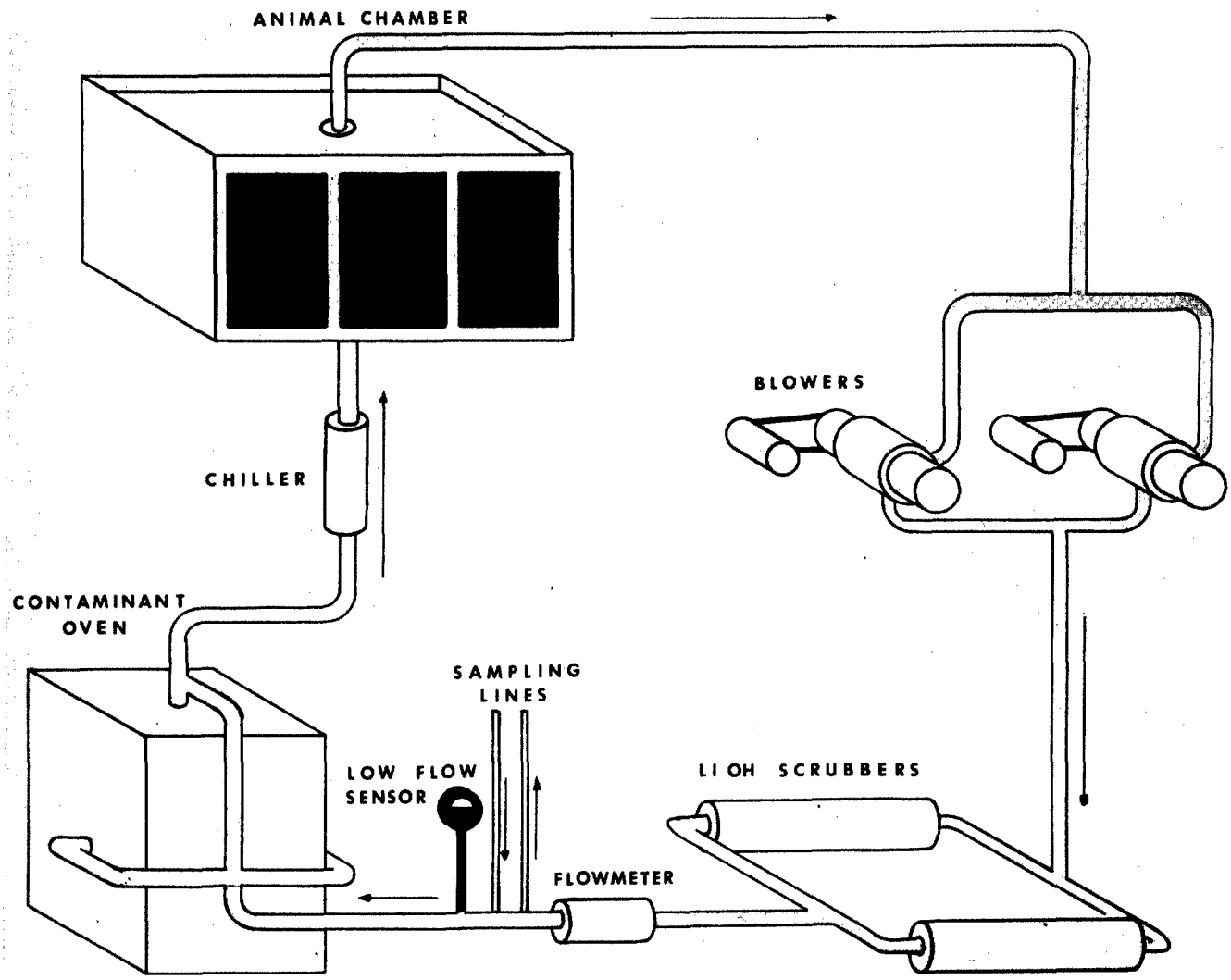


Figure 23. Schematic Diagram - Closed-loop Life Support System

one purpose or another. An occasional test group was the "positive control" animal complement which was housed in a life support loop without, however, being exposed to gas-off products.

Body weights are recorded, pre- and immediate post-exposure, as well as at weekly intervals prior to sacrifice. The standard sacrifice schedule involves five animals of each species at one-, two-, three-, and four-week post-exposure periods, at which time the weights of individual excised organs of the rats are determined. Control animals are treated in a like manner.

Test materials are weighed prior to being placed in the ovens of the life support loop and again at the conclusion of exposure. Thus, any weight change undergone by any test material within the group is known for that period of exposure. What is not known, however, is whether the change occurred primarily at the beginning of exposure, or whether there was gradual change throughout the period of test. A few materials tested thus far exhibited weight gains (probably from water vapor absorption); a few showed rather large weight losses. But approximately 67% of all materials showed less than 1% loss.

Mention was made in the last annual report of results with group "F" Apollo materials; wherein a marked weight loss was exhibited by rats at the last two post-exposure observations. The "F" group was, therefore, rescheduled in additional experiments. These (indicated in Table I) showed no detectable effect in either rats or mice, but results do not necessarily indicate an absolute "no effect" response. All materials comprising the latter test group did not duplicate the same batch source of those in the original "F" group.

A summary statement concerning the results of the Apollo screening tests can be made with some degree of certainty, however, providing that the limitations of the methods of assessment of effect are recognized. The critical criteria for assessment, i.e., body weight gain, organ weights, organ-to-body weight ratios, and gross pathologic scan at necropsy with some selected examination for histopathology, indicate lack of demonstrable effect that can be ascribed to toxic properties of gas-off products of the Apollo materials tested.

Greater detail relative to conditions of tests, control of environmental parameters, and results of the Apollo screening program are documented elsewhere (References 2,5).

3. Special Studies

Listed under this category heading are various experimental and control studies of a specialized nature, i.e., non-continuing, short-term, or one of a kind.

a. Most prominent was a study involving assessment of inhalation toxicity of ethylene glycol vapors (Table I, #193 and #194). Because of the interest in, and use of, ethylene glycol as a heat transfer medium in aerospace applications, we investigated the effects of this agent in animal exposures to saturated vapor concentrations in a 5 psia 100% oxygen atmosphere at a nominal temperature of 73. 2F. Under these conditions, ethylene glycol has a saturation concentration of 254 mg/M^3, this being equivalent to 100 ppm at 760 mm Hg.

Protocol called for exposure of six animal species (mice, rats, guinea pigs, rabbits, beagle dogs, and Rhesus monkeys) for an initial period of 14 days. If, at the end of this time, no overt symptoms of toxicity appeared, the study was to continue through 60 days. However, cessation of experiments with high oxygen atmospheres (for reasons mentioned previously) necessarily halted the glycol experiment prematurely; thus, the maximum term of exposure for any species was 21 days. Control animals for those counterparts on experiments were housed in an ambient environmental situation and monitored by the same criteria used to assess an effect in the experimentals.

The criteria for assessment of toxicity and/or altered physiologic response were:

(1) Clinical chemistry determinations and hematologic measurements listed in Table III; also included were measurements for blood urea nitrogen (BUN), creatinine, and ethylene glycol blood levels.

(2) Behavioral performance studies with mice and Rhesus monkeys were performed; the ability of these species to perform would, hopefully, detect any effect of borderline toxicity involved by glycol inhalation.

(3) Body weight changes were followed, and necropsy material was utilized for organ-to-body weight calculation as well as for gross and histopathologic evaluation.

(4) Any ensuing mortality was taken into account.

Description of the methodology for generation and control of ethylene glycol vapors and analysis of dome concentrations, as well as other details pertinent to the study, was presented at the 3rd Annual Conference and is documented in the Proceedings of same (Reference 12). These are also mentioned in this report under Section II: Facilities, Chemistry.

Results of exposure to this relatively high concentration of ethylene glycol indicate significant toxicity for some animal species and little or none for others. Body weight changes (losses) indicated a toxicity, or at

least an anorexic effect, in both rats and guinea pigs. Limited studies on quantitative determination of lung water and solids content of these two species also pointed to an edemagenic response of lung tissue. Histopathologic findings showed mice lungs to be similarly affected. BUN determinations and comparison with control values indicated toxicity in rabbits (significantly elevated values), but dogs and monkeys did not show this response. Examination of all criteria indicated that monkeys and dogs were not adversely affected by exposure to continuous saturated vapor concentrations of ethylene glycol (mean = 264 mg/M^3) for 21 days. Interpretation of the pathologic findings was presented at this year's conference (Reference 11).

Note that although the species of animals highest on the phylogenetic tree did not show detectable effects as a result of ethylene glycol exposure, this does not necessarily sanction similar human exposure. Evidence from the literature indicates that man is considerably more susceptible to the effects of the agent than are lower animals, at least by routes of entry other than inhalation. Little is known concerning inhalation toxicology of ethylene glycol.

b. Special studies were conducted in Thomas Dome 3 with a group of monkeys trained at Holloman Air Force Base. Background information concerning the use and handling of these animals can be found elsewhere (Reference 13). Briefly reiterated, the psychopharmacological studies with trained monkeys are programmed by Air Force personnel, while THRU personnel are responsible for dome maintenance and control of exposure atmospheres. The animals are negatively reinforced on a continuous avoidance task and discrete avoidance tasks involving visual or auditory cues. This series of experiments is not included in Table I, but did involve services of THRU personnel during the period of this report. The studies referred to were: (1) a 90-day continuous exposure to a Threshold Limit Value (TLV) of ozone (0.2 mg/M^3), (2) initiation of a 90-day exposure to a TLV of NO_2 (9.4 mg/M^3), and (3) an 18-day exposure to a saturated concentration of ethylene glycol vapor (same concentration as that cited above for experiment #193).

All experiments were conducted at simulated altitude (5 psia or 260 mm Hg), with the first two having, in addition to the contaminants, a breathing atmosphere of 68% O_2 and 32% N_2; the latter involved use of a 100% O_2 atmosphere in addition to glycol vapors. The first began during the period covered by the last annual report, while the remaining two began during the period reported here. The NO_2 study was discontinued after 38 exposure days, while the last was discontinued after 18 days for reasons pointed out above.

c. Several additional experiments were conducted using NO_2 as a model contaminant. One of these (#183) was designed to test the effects of a mixed gas atmosphere (68% O_2 and 32% N_2) on the response of dogs, rats, and

mice exposed for 14 days to 70 mg/M^3 of NO_2. The others (#188 and #190) were identical in purpose and protocol, except that the O_2 concentration was maintained at 100%. Experiment #188 was prematurely terminated because of an unavoidable low contaminant concentration. The deficiency was noted during day two of exposure; therefore, the dogs were removed and saved for later acute, range finding exposures in propellant toxicity screening. The rodents were utilized by continuing the NO_2 exposure to teach laboratory personnel the methodology for quantitative determinations of lung fluid and solid content resulting from exposure to pulmonary irritants.

Other experiments were interjected at various times to demonstrate (1) rather subtle pulmonary inflammatory response to agents, and (2) to emphasize the necessity of minimizing the chances of introducing pulmonary infections, particularly in rats. The subject of "healthy" rats is one of great concern, requiring continuous surveillance and effort to reduce all possible sources and manipulations that can introduce infection and/or conditions which exacerbate latent infection.

Results of the two experiments with contaminant NO_2 in concentrations of 70 mg/M^3 for 14 continuous exposure days indicate that this agent is significantly more toxic in 100% O_2 at 5 psia than in 68% O_2 at the same reduced pressure. This response contrasts with that previously determined for another deep-lung irritant, namely, ozone. A higher O_2 concentration reduced ensuing mortality from ozone exposure (Reference 9). This finding further substantiates the inference from other research that NO_2 and ozone, contradictory to classic interpretation, appear to have basically different mechanisms of action (Reference 4).

d. The abatement of studies in the Altitude Facility, or in any other area, involving atmospheres of O_2 concentration above ambient, provided impetus for a control study for collection of baseline data. A truly ambient control had not been done prior to this time because of the great demand for dome experimentation, and control animals had, of necessity, been housed at ambient conditions in the animal room.

Accordingly, a control study comparing baseline data from animals in a Thomas Dome and their counterparts in normal housing was begun in February and still continues (Table I, #196, and #197). The animals within the dome comprise a complement identical in species and number to that called for in routine experimental protocol. The animals are handled and sampled in an identical manner to that employed under previous situations. The collection of control data will continue until the dome is no longer available for this use, i.e., until modifications for new fire detection and extinguishing system begin.

e. As an aftermath of the tragic events associated with O_2 atmospheres this year, various directives have required modifications of all altitude simulators and/or chambers. The modifications will be effected prior to the resumption of activities in the THRU Altitude Facility. Among the directives was one concerning the fire extinguishing capabilities of systems of a Thomas Dome. To test certain dome modifications, including fire extinguishing equipment, experiments were planned for igniting fires in Dome 4 under specific conditions of test. These were to be fire tests under conditions of 100% oxygen, one test at altitude (250 mm Hg) and a second at ambient (745 mm Hg) pressure. The first experiment (altitude) was designed to simulate the presence of a clothed dome entrant. For this purpose THRU personnel fabricated a manikin from a frame of chicken wire wrapped with plaster of paris impregnated gauze strips. The manikin was clothed in fire retardant coveralls weighing approximately 1 kilogram, rubber boots, communications headset, and an oxygen mask with its flexible hose. Ignition was propagated by a nichrome wire positioned against the inner portion of one leg of the coverall. The second experiment was planned and put into effect based on the outcome of the first. For this, at 745 mm Hg pressure, a strip of fire retardant treated cotton cloth weighing approximately 200 grams was used as the limit of burnable material. Protocol called for ignition at the bottom of the hanging strip in the same manner as that for the clothed manikin in the altitude experiment.

THRU personnel cooperated with AMRL representatives in drawing up a detailed protocol covering all conceivable emergencies that could arise during the course of tests. Fire, police, ground safety, and medical officers were contacted, in order that their particular capabilities could be utilized and written into the test protocol. Plans were made for several briefing sessions, as well as rehearsals, with all personnel involved in the tests.

Results of the fire tests, which were conducted 13 May 1967 have been reported previously and are herewith reiterated. A flash nap fire occurred in Test 1 immediately after ignition and spread over the whole coverall. The normal, slower burning fire proceeded up the rear of the leg and reached the right buttock area of the coverall before extinguishment. The manikin was scorched in this area. After the fire and drying, the manikin and burnables were examined with the following observations:

The areas over which the flash nap fire had spread were easily identified by very small black spots over the surface. The exterior of the coverall was completely covered by these, as was the interior, except for those areas which were in direct contact with the manikin which did not appear to have experienced a nap fire. At isolated spots in the nap fire area, foci of normal burning appeared to have initiated. These were areas of from 1-10 mm in diameter where browning or charring had occurred to some degree. These

focal fires were all extinguished by the water deluge. Two residues of focal burning were discovered on the inside of the coverall, but these too were extinguished by the water deluge.

Neither the oxygen mask, flexible hose, nor headset appeared to have been affected in any way. The beta cloth leg was unmarked as was the biply underwear stockinette beneath it except for a small scorched area, 5 mm long, at the bottom edge. This had been tucked into the left boot which itself showed no sign of scorching. The right boot immediately below the ignition point was slightly burned at the top, but this did not register as a loss in weight. The stockinette was soaking wet after the water deluge, but it is not certain whether this was caused by dripping from the top of the leg or transmission through the beta cloth.

Color moving picture and audio tape records were kept of the various aspects of the fire. The moving pictures were taken at speeds of 24, 64, 500, and 1000 frames per second. From these records, estimates were made of the elapsed time for various operations and burning times. The pre- and post-fire dry weights of the burnables in the two tests, along with the percent losses, were calculated.

As a result of the fire tests there has been some agreement for reactivation of Dome 4 (the one used for the test) after the addition of automatic fire detection and water activation equipment. Specific design parameters will be established as well.

B. AMBIENT FACILITY EXPERIMENTS

All experimental and/or control studies conducted in the Ambient Facility are listed in Table II. This does not include, however, studies under ambient conditions which served as controls for experiments in the Altitude Facility domes, inasmuch as they have been included with their respective experimental counterparts.

1. Monomethylhydrazine (MMH) Studies

Examination of Table II shows that the great majority of experimental work in the Ambient Facility involved inhalation exposures of either mice, rats, dogs, or squirrel monkeys to MMH. These were conducted in the pilot chamber units or in Rochester Chamber B. Exposures are chiefly concerned with the accumulation of data with which LC_{50}'s or estimates of LC_{50}'s can be made for several animal species. Thus, 15- and 30-minute, and hour, two-hour, and four-hour LC_{50}'s or estimates thereof can give some basis for judicious application toward interpretation of emergency exposure limits for man. Secondarily, some application of toxicologic disciplines will permit a better understanding of the nature of the effects upon biological systems.

Considerable numbers of pilot studies were conducted prior to establishing reliable and accurate systems for the generation and analysis of MMH vapors. Both the Auto-Analyzer and Billion-Aire were utilized to measure MMH concentrations, with ultimate use of the latter instrument almost exclusively as the one of choice. After having solved the inherent difficulties and inconsistencies that plagued initial trials, the MMH exposures were begun and continued with regularity. These progressed from various time-exposures with mice in the 30 liter pilot chambers to exposures of large animals in a Rochester Chamber. All are shown with their respective conditions of testing in Table II.

Certain modifications of the Rochester Chamber were required before good MMH concentration control was obtained. These modifications were mentioned above under Section II, Engineering Facility. Concentration distribution within the chamber was checked for uniformity and found to vary no more than 2% between eight sampling points. Similarly, the system was tested to determine reproducibility of response by comparison of mouse mortality from test to test in both the Rochester Chamber and the small pilot chamber using a given MMH concentration. Certain other influencing factors were checked in order to ascertain their effect upon MMH concentration and upon resulting animal mortalities. Two such variables were humidity and chamber air flow, both of which were characterized as to limitations that they might impose.

Sufficient MMH exposure data have been obtained for calculation of mouse and rat LC_{50}'s, as well as for rough estimation of dog and squirrel monkey LC_{50}'s. Calculation and/or estimates were based upon probit analyses which utilized either a linear estimate of least squares fit or graphic plots. LC_{50} values and their respective 95% Confidence Limits are presented in Table IV. Two sets of values are shown for rats; those designated as "new data" were calculated from the most recent experiments wherein analytical measurement of MMH was more sophisticated and reliable. Thus, these values are considered to be more representative of toxicity for rats than are values shown for "old data". It is pointed out that the Confidence Limits indicated for dogs and monkeys are only estimates, inasmuch as too few animals were utilized to delineate precise levels.

Because of the limited number of dogs and primates available, there has not been any attempt to precisely calculate LC_{50}'s. The design of exposures, however, is meant to give at least some information relative to rough estimation of the 50% lethal concentration for given periods of exposure. Even so, it is considered more important to gather as much information as possible concerning the nature of MMH toxicity upon organ systems. To this end the dog and large primates are exemplary because sufficient blood can be taken for chemical and hematologic measurements, as well as serving to monitor symptomatology of toxic manifestations.

Accordingly, clinical hematology determinations for dogs have been selected to monitor effects induced by MMH, depending upon the expected results of exposure. Thus, if the MMH concentration is expected to produce mortality, a "series A" profile consisting of 20 determinations on pre- and post-exposure blood is called for. If survival is expected, then protocol calls for a "series B" profile which measures 12 factors at weekly intervals and a "series C" of 6 determinations in between until the blood picture returns to normal. Series A consists of determinations shown in Table III, as well as those for blood urea nitrogen, plasma hemoglobin, prothrombin time, bilirubin, and creatinine. Series B profile includes determinations for (1) hematocrit, (2) hemoglobin, (3) red blood cell count, (4) white blood cell count, (5) bilirubin, (6) total protein, (7) albumin, (8) blood urea nitrogen, (9) plasma hemoglobin, (10) reticulocyte count, (11) prothrombin time, and (12) creatinine. Those included in the series C are numbers 1, 2, 3, 4, 9, and 10 listed above.

Rather consistent findings in the non-fatal exposures of dogs show a picture of hemolytic anemia; the hematocrits, erythrocyte counts, and hemoglobins decline steadily for several days post-exposure, remain somewhat lowered for varying periods, and then rise toward normal. More often than not, the reticulocyte counts rise during the period of maximum decline of the above parameters, indicating a state of repairative process.

A steep slope in dose-response curve is exhibited by all species tested with MMH thus far. This undoubtedly relates to findings wherein some dogs experience maximum nonlethal exposures and show minimal symptomatology of central nervous stimulation, yet exhibit a marked picture of anemia; whereas others show severe symptoms of overt stimulation but little transitory hemolytic anemia. There would appear to be no direct correlation between degree of cell hemolysis and degree of central nervous response.

Results of tests with squirrel monkeys have not indicated noteworthy differences in symptomatologic response of this species. Hematologic measurements are not being made, due to the small size of the animals and the difficulty in taking enough blood for analysis. Exposure of several Rhesus monkeys, however, is planned in order to conduct hematologic studies comparable to those being determined for the dog.

In all instances of mortality of large animal species resulting from MMH exposure, the subjects are necropsied for gross and histopathologic examination. Additionally, some exposures are conducted with certain species in order to study sequential pathogenesis, if any.

2. In addition to control studies in the Altitude Facility (mentioned above), others of varied nature have been conducted and are most appropriately categorized under Ambient Facility. One considered noteworthy concerns a study comparison of data collection from necropsied animals. These were

prompted as a result of the several recognized inconsistencies in handling rodents for the Apollo screening studies. Analysis of organ/body weight data from the toxicity screening tests of various groups of Apollo space cabin construction materials revealed that meaningful interpretation of results was deficient. Often the statistical treatment of data indicated an effect; yet, further scrutiny revealed that what might have been an effect, by comparison with control data, was merely appearing out of line because the control data were abnormal. Similarly, statistical analysis of the data often showed significant differences between control and experimental groups, but the differences were never consistent for a pattern or trend to be evident as would be expected in a true toxic response of organ or multiple organ injury.

Accordingly, studies for comparison of techniques and methodology applicable to Apollo screening studies are underway. These will be continued for some time in order to have a complete evaluation during various growth phases of the animals. The study involves comparison of data collection in order to develop a standardized procedure which will essentially "normalize" data as much as possible, because the many inherent errors can either be smoothed out, reduced, or at least duplicated for both controls and experimentals. Comparison is being made for the following parameters:

a. Rats that have neither been fasted nor exsanguinated prior to sacrifice with an overdose of barbiturates.

b. Rats that have been fasted prior to sacrifice by exsanguination.

c. Rats that have not been fasted, but have been sacrificed by exsanguination.

d. Rats that have been fasted, but have been sacrificed with an overdose of barbiturates rather than exsanguination.

Good toxicologic practice recognizes the second procedure listed above as the most reliable for obtaining consistent assessment of organ-to-body weight change as a result of toxicity. In this manner, the animal, whether a control with healthy appetite, or an experimental with good to poor appetite, has had his body weight "normalized" by fasting over the night before sacrifice. Similarly, exsanguination tends to "normalize" organ weight by reducing the variation of blood engorgement during euthanasia. One case in point, as an example of induced variability, is the fact that fully fed rats prior to sacrifice have larger livers than rats fasted overnight. The digestion process tends to increase liver size significantly.

This evaluation study is considered to be mandatory from the point of view of conducting the best possible screening test for potential toxicity of gas-off products of the Apollo cabin construction materials, as well as for the MOL screening studies to begin in the future.

TABLE I
SUMMARY OF EXPERIMENTS
ALTITUDE FACILITY*

Experiment** & Control (C) Number	Experiment Dates	Days Duration	Experimental Contaminant	Concentration (mg/M^3)	Experimental Conditions mm Hg; Gas	Animal*** Species
167 ≠	2 May 1966 - 5 Jan. 1967	248	None	--	260; 68% O_2 & 32% N_2	M, R, D, My
168 ≠ (C)	2 May 1966 - 5 Jan. 1967	248	None	--	AR & AC	M, R, D, My
176-1 ≠	12 July - 9 Sept. '66	60	Apollo "J"	--	260; O_2	M, R
176-2 ≠ (C)	12 July - 9 Sept. '66	60	None	--	260; O_2	M, R
176-3 ≠ (C)	12 July - 9 Sept. '66	60	None	--	AR & AC	M, R
183 ≠	24 Aug. - 7 Sept. '66	14	NO_2	70	260; 68% O_2 & 32% N_2	M, R, D
184 ≠ (C)	24 Aug. - 7 Sept. '66	14	None	--	AR & AC	M, R, D,
185-1	22 Sept. - 29 Sept. '66	7	Apollo "F"	--	260; O_2	M, R
185-2	22 Sept. - 29 Sept. '66	7	Apollo "F_1"	--	260; O_2	M, R
185-3	22 Sept. - 29 Sept. '66	7	Apollo "F_2"	--	260; O_2	M, R
185-4 (C)	22 Sept. - 29 Sept. '66	7	None	--	260; O_2	M, R
185-5 (C)	22 Sept. - 29 Sept. '66	7	None	--	AR & AC	M, R

TABLE I, Continued

Experiment** & Control (C) Number	Experiment Dates	Days Duration	Experimental Contaminant	Concentration (mg/M^3)	Experimental Conditions mm Hg; Gas	Animal*** Species
186-1	11 Oct. - 18 Oct. '66	7	Apollo "F_{1B}"	--	260; O_2	M,R
186-2	11 Oct. - 18 Oct. '66	7	Apollo "F_{1A}"	--	260; O_2	M,R
186-3	11 Oct. - 18 Oct. '66	7	Apollo "F_{1A1}"	--	260; O_2	M,R
186-4 (C)	11 Oct. - 18 Oct. '66	7	None	--	260; O_2	M,R
186-5 (C)	11 Oct. - 18 Oct. '66	7	None	--	AR & AC	M,R
187-1	11 Nov. - 18 Nov. '66	7	Apollo "K"	--	260; O_2	M,R
187-2 (C)	11 Nov. - 18 Nov. '66	7	None	--	260; O_2	M,R
187-3 (C)	11 Nov. - 18 Nov. '66	7	None	--	260; O_2	M,R
187-4 (C)	11 Nov. - 18 Nov. '66	7	None	--	260; O_2	M,R
187-5 (C)	11 Nov. - 18 Nov. '66	7	None	--	AR & AC	M,R
188 ≠	15 Nov. - 16 Nov. '66	1	NO_2	70	260; O_2	M,R,D
189 (C)	15 Nov. - 16 Nov. '66	1	None	--	AR & AC	M,R,D
190	8 Dec. - 22 Dec. '66	14	NO_2	70	260; O_2	M,R,D
191 (C)	8 Dec. - 22 Dec. '66	14	None	--	AR & AC	M,R,D

TABLE I, Continued

Experiment** & Control (C) Number	Experiment Dates	Days Duration	Experimental Contaminant	Concentration (mg/M^3)	Experimental Conditions mm Hg; Gas	Animal*** Species
192	14 Dec. - 21 Dec. '66	7	None	--	260; O_2	R
193	6 Jan. - 27 Jan. '67	21	Ethylene glycol	254	260; O_2	M, R, D, My Rb ϕ G.P. ϕ
194 (C)	6 Jan. - 27 Jan. '67	21	None	--	AR & AC	M, R, D, My Rb ϕ, G.P. ϕ
195-1	18 Jan. - 25 Jan. '67	7	Apollo "L"	--	260; O_2	M, R
195-2	18 Jan. - 25 Jan. '67	7	Apollo "M"	--	260; O_2	M, R
195-3 (C)	18 Jan. - 25 Jan. '67	7	None	--	260; O_2	M, R
195-4 (C)	18 Jan. - 25 Jan. '67	7	None	--	260; O_2	M, R
195-5 (C)	18 Jan. - 25 Jan. '67	7	None	--	AR & AC	M, R
196	17 Feb. - Current	-	None	--	700; Air	M, R, D, My
197	17 Feb. - Current	-	None	--	AR & AC	M, R, D, My

* Thomas Domes, unless otherwise indicated, e.g., AR = Animal Room & AC = Ambient Conditions for experimental controls.
** Sequential continuation of numerical system used under previous report (AMRL-TR-66-177).
*** M = Mice, R = Rats, D = Beagle dogs, and My = Rhesus monkeys
$\neq$ Experimental and control studies began under old contract and completed under current contract.
$\not\equiv$ Aborted due to loss of contaminant control.
ϕ Additional animal species, Rb = rabbits and G.P. = guinea pigs.

TABLE II
SUMMARY OF EXPERIMENTS
AMBIENT FACILITY*

Expt. No.	Tests per Expt.	Expt. Dates	Duration	Expt'l. Agent	Concentration (ppm) Actual Mean	Expt'l. Purpose & Facility	Animal Species**
182-1 to 182-40	1	1 Sept. '66 - 19 Dec. '66	30 min, 1, 2, & 4 hrs.	MMH***	varied	Pilot Studies (P. C.)	M
182-41	4	6 Jan. '67	30 min.	MMH	143, 135 142, 143	LC_{50}+(P. C.)	M
182-42	2	12 Jan. '67	30 min.	MMH	205, 200	LC_{50}+(P. C.)	M
182-43	2	13 Jan. '67	30 min.	MMH	250, 250	LC_{50}+(P. C.)	M
182-44	2	16 Jan. '67	30 min.	MMH	322, 324	LC_{50}+(P. C.)	M
182-46	2	18 Jan. '67	30 min.	MMH	380, 345	LC_{50}+(P. C.)	M
182-47	2	20 Jan. '67	1 hr.	MMH	155, 155	LC_{50}+(P. C.)	M
182-48	1	27 Jan. '67	1 hr.	MMH	180	LC_{50}+(P. C.)	M
182-49	1	30 Jan. '67	1 hr.	MMH	200	LC_{50}+(P. C.)	M
182-50	2	30 Jan. '67	1 hr.	MMH	120, 120	LC_{50}+(P. C.)	M
182-51	2	1 Feb. '67	1 hr.	MMH	85, 70	LC_{50}+(P. C.)	M

TABLE II, Continued

Expt. No.	Tests per Expt.	Expt. Dates	Duration	Expt'l. Agent	Concentration (ppm) Actual Mean	Expt'l. Purpose & Facility	Animal Species**
182-52	2	2 Feb. '67	1 hr.	MMH	113, 105	LC_{50}^{+}(P. C.)	M
182-53	2	6 Feb. '67	1 hr.	MMH	100, 98	LC_{50}^{+}(P. C.)	M
182-54	2	7 Feb. '67	1 hr.	MMH	145, 140	LC_{50}^{+}(P. C.)	M
182-57	2	13 Feb. '67	2 hr.	MMH	83, 85	LC_{50}^{+}(P. C.)	M
182-58	2	14 Feb. '67	2 hr.	MMH	58, 60	LC_{50}^{+}(P. C.)	M
182-59	2	16 Feb. '67	2 hr.	MMH	105, 108	LC_{50}^{+}(P. C.)	M
182-60	2	20 Feb. '67	2 hr	MMH	68, 68	LC_{50}^{+}(P. C.)	M
182-61	2	21 Feb. '67	2 hr.	MMH	120, 128	LC_{50}^{+}(P. C.)	M
182-62	2	23 Feb. '67	4 hr.	MMH	26, 25	LC_{50}^{+}(P. C.)	M
182-63	2	24 Feb. '67	4 hr.	MMH	50, 50	LC_{50}^{+}(P. C.)	M
182-64	2	28 Feb. '67	4 hr.	MMH	83, 83	LC_{50}^{+}(P. C.)	M
182-65	2	2 Mar. '67	4 hr.	MMH	68, 66	LC_{50}^{+}(P. C.)	M
182-66	2	4 Mar. '67	4 hr.	MMH	63, 60	LC_{50}^{+}(P. C.)	M
182-67	2	4 Mar. '67	4 hr.	MMH	55, 55	LC_{50}^{+}(P. C.)	M

TABLE II, Continued

Expt. No.	Tests per Expt.	Expt. Dates	Duration	Expt'l. Agent	Concentration (ppm) Actual Mean	Expt'l. Purpose & Facility	Animal Species**
182-68	2	7 Mar. '67	4 hr.	MMH	63, 63	LC_{50}^{+}(P. C.)	M
198 ≠	2	12 April '67 - 31 May '67	49 days	None	--	Control Studies	R, D,
199 ∅	4	28 April '67 - Present	Continuing	None	--	Control Studies	R
200-1	1	17 Mar. '67	1 hr.	MMH	103	LC_{50}^{++}(R. C.)	D
200-2	1	20 Mar. '67	1 hr.	MMH	90	LC_{50}^{++}(R. C.)	D
200-3	1	22 Mar. '67	1 hr.	MMH	106	LC_{50}^{++}(R. C.)	D
200-4	1	4 April '67	1 hr.	MMH	93	LC_{50}^{++}(R. C.)	D
200-5	1	12 April '67	1 hr.	MMH	93	LC_{50}^{++}(R. C.)	D
200-6	1	18 April '67	30 min.	MMH	178	LC_{50}^{++}(R. C.)	D
200-7	1	19 April '67	30 min.	MMH	190	LC_{50}^{++}(R. C.)	D
200-8	1	24 April '67	30 min.	MMH	200	LC_{50}^{++}(R. C.)	D
200-9	1	28 April '67	30 min.	MMH	200	LC_{50}^{++}(R. C.)	D
200-10	1	2 May '67	30 min.	MMH	190	LC_{50}^{++}(R. C.)	D
200-11	1	5 May '67	30 min.	MMH	180	LC_{50}^{++}(R. C.)	D

TABLE II, Continued

Expt. No.	Tests per Expt.	Expt. Dates	Duration	Expt'l. Agent	Concentration (ppm) Actual Mean	Expt'l. Purpose & Facility	Animal Species**
200-12	1	16 May '67	30 min.	MMH	188	LC_{50}^{++}(R.C.)	D
200-13	1	18 May '67	15 min.	MMH	380	LC_{50}^{++}(R.C.)	D
200-14	1	22 May '67	15 min.	MMH	400	LC_{50}^{++}(R.C.)	D
200-15	1	23 May '67	15 min.	MMH	390	LC_{50}^{++}(R.C.)	D
200-16	1	24 May '67	15 min.	MMH	380	LC_{50}^{++}(R.C.)	D
200-17	1	26 May '67	15 min.	MMH	400	LC_{50}^{++}(R.C.)	D
200-18	1	31 May '67	15 min.	MMH	390	LC_{50}^{++}(R.C.)	D
200-19	1	2 June '67	15 min.	MMH	400	LC_{50}^{++}(R.C.)	D
200-20	1	5 June '67	15 min.	MMH	400	LC_{50}^{++}(R.C.)	D
200-21	1	7 June '67	15 min.	MMH	400	LC_{50}^{++}(R.C.)	D
201-1	1	5 June '67	1 hr.	MMH	150	LC_{50}^{++}(R.C.)	My
201-2	1	6 June '67	1 hr.	MMH	150	LC_{50}^{++}(R.C.)	My
201-3	1	12 June '67	1 hr.	MMH	130	LC_{50}^{++}(R.C.)	My
201-4	1	13 June '67	1 hr.	MMH	110	LC_{50}^{++}(R.C.)	My
201-5	1	14 June '67	1 hr.	MMH	75	LC_{50}^{++}(R.C.)	My

TABLE II, Continued

Expt. No.	Tests per Expt.	Expt. Dates	Duration	Expt'l. Agent	Concentration (ppm) Actual Mean	Expt'l. Purpose & Facility	Animal Species**
201-6	1	15 June '67	1 hr.	MMH	85	LC_{50}^{++}(R.C.)	My
201-7	1	16 June '67	1 hr.	MMH	75	LC_{50}^{++}(R.C.)	My
201-8	1	19 June '67	1 hr.	MMH	85	LC_{50}^{++}(R.C.)	My
202	-	20 June '67	15 min., 30 min., 1 hr., 2 hr., & 4 hr.	MMH	varied	LC_{50}^{+}(R.C.)	R

* Refers to Rochester Type Chambers (R.C.), 30 litre Pilot Chamber (P.C.), or animal room (A.R.).
** M = Mice, R = Rats, D = Beagle dogs, My = Squirrel Monkeys
*** Monomethylhydrazine
\+ Data to be used for calculating LC_{50}'s (concentration which produces 50% mortality from a given time exposure).
++ Data to be used for an LC_{50} estimate (too few animals and exposures for accurate LC_{50}).
≠ Control study, ambient conditions, new animal housing facility.
ø Control study, ambient conditions, evaluation of sacrifice techniques.

TABLE III

CLINICAL LABORATORY TESTS PERFORMED

BIWEEKLY ON BEAGLE DOGS AND RHESUS MONKEYS

HEMATOLOGY	CHEMISTRY
Total WBC (Cells/MM^3)	Sodium (MEQ/L)
Differential (Per 100 Cells)	Potassium (MEQ/L)
Total RBC (Million Cells/MM^3)	Calcium (MEQ/L)
Hemoglobin (GM%)	Total Protein (GM%)
Hematocrit (VOL.%)	Albumin (GM%)
	S-GPT (RF Units)
	S-GOT (RF Units)
	Alkaline P-Tase (KBR Units)
	Total Phosphorus (MG%)
	LDH (CW Units)

TABLE IV

Calculated and Estimated* LC_{50}'s** With

95% Confidence Limits for MMH (ppm)

SPECIES	EXPOSURE TIME (HOURS) 1/4	1/2	1	2	4
Rat (♂)					
New Data	?	410 (365-465)	245 (197-310)	127 (115-140)	79 (71-86)
Old Data	?	525 (360-800)	190 (140-265)	104 (91-120)	34 (28-42)
Mouse (♂)	?	285 (232-338)	124 (93-155)	93 (76-111)	66 (57-74)
Beagle* (♂and♀)	390 (370-410)	193 (185-197)	96 (92-99)	--	--
Sq. Monkey* (♀)	340 (315-365)	146 (135-157)	84 (81-87)	--	--

**50% Lethal Concentration 24 hours post-exposure

SECTION IV

ANNUAL CONFERENCE AND MEETINGS

A. ANNUAL TOXICOLOGY CONFERENCE

The 3rd Annual Conference on Atmospheric Contamination in Confined Spaces was held 9, 10, and 11 May 1967. Civilian and military scientist attendees numbered 181, a small increase over past years. A total of five sessions made up the Conference agenda this year, these being (1) Toxicological Evaluation of Atmospheres and Contaminants, (2) Histopathological Evidences of Toxicity, (3) Evaluation of Cabin Materials, (4) Instruments and Detection Techniques, and (5) Measurement of Behavioral Responses. Approximately 13 of the 28 technical papers concerned work which was either a direct outgrowth or by-product of THRU studies. Five of the THRU staff made presentations.

B. SHORT COURSES AND TECHNICAL MEETINGS

During the period covered by this report, various individuals of THRU attended meetings. Personnel of the Chemistry section attended schools of instruction in the Technicon Auto-Analyzer, Bendix TOF Mass Spectrometer, and the Perkin-Elmer short course in gas chromatograph - infrared. Technical meetings included the Pittsburgh Conference on Analytical Chemistry and the Fourth International Symposium on Gas Chromatography.

Toxicology personnel attended and presented papers at the annual meetings of the Society of Toxicology and the American Industrial Hygiene Association. Also attended were meetings of the Industrial Hygiene Foundation and the Society for Experimental Biology and Medicine.

Engineering oriented meetings attended were the ISA Instrumentation Maintenance Symposium and the Society of Instrumentation and Automation.

Two individuals from THRU attended a Fire Hazards and Extinguishment Conference at Brooks Air Force Base, Texas. This informal conference was an outgrowth of the Aerospace Medical Division's extensive investigation of fire extinguishing systems for use in hypo- and hyper-baric chambers.

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
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13. ABSTRACT This report reviews the activities of the Toxic Hazards Research Unit (THRU) since August, 1966. Included is a brief resume' of various facility and equipment design modifications which have occurred; in addition, research accomplishments are reported and pertain to all experiments conducted during the year, including those which were begun during the period of the last annual report. Facility modifications have primarily been restricted to the Altitude Facility with the view in mind to assume greater safety against fire hazards in environments of high oxygen concentrations. Toward this end, much has been accomplished in design and test of fire extinguishing systems. Research activities were curtailed by the restrictions placed upon use of environmental atmospheres containing O_2 concentrations greater than ambient, however, various control studies were designed and conducted to take advantage of the abated experimental programs. An 8-month study of the effects of a mixed gas (O_2-N_2) atmosphere at 5 psia indicated some adverse findings. The Apollo materials toxicity screening tests have continued with little or no evidence of toxicity exhibited by their gas-off products. Special studies, including one with ethylene glycol, were conducted and the findings are reported herein. Ambient Facility experiments have mainly comprised work with monomethylhydrazine (MMH) and certain control studies to delineate improvement of animal sacrifice techniques. MMH exposures of various time periods are reported for mice, rats, dogs and monkeys.

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